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SHORT-LATENCY CROSSED SPINAL RESPONSES IN THE HUMAN SOLEUS MUSCLE DURING SITTING AND WALKING

by

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ENGLISH SUMMARY

Interlimb reflex pathways were initially investigated by Sherrington (1910), who demonstrated that the crossed extensor reflex was evident in the cat and suggested that it may have a functional role. This work formed the basis for future (interlimb) reflex research on the human and the cat. Due to the inability to perform invasive studies in the human, the cat is often used as a model for understanding neural pathways. However, the cat and the human have many differences (for example: quadrupedal vs. bipedal). In the human there is an increased reliance on the ankle extensors compared to the cat during walking. Therefore, although research in the cat shows limited responses in contralateral ankle extensor efferents following stimulation of the ipsilateral ankle extensor nerve afferents, there are possibly differences in the reliance of interlimb feedback from the ankle extensors in the human. The aims of this PhD were to investigate (i) if crossed reflexes are present in sitting and a functional task as human walking following electrical stimulation (**Study I and III**) (ii) how they are modulated (**Study III–V**) and (iii) the likely pathways and nerve fibres involved (**Study I–III**).

From the current thesis it can be concluded that short latency interlimb reflexes are observed from the ipsilateral tibial nerve to the contralateral soleus and are: (i) inhibitory and observed in sitting and walking (ii) modulated by supraspinal areas due to the phase dependence and alterations in patients with stroke (iii) likely mediated by large diameter ipsilateral muscle/tendon afferents.

The current thesis suggests that commissural interneurons are present in the human with input from ipsilateral ankle extensor muscle or tendon afferents to the contralateral soleus. It is proposed that the stimulus to the ipsilateral tibial nerve may indicate a mechanical disturbance to the ipsilateral ankle extensors with the inhibition initially halting the contralateral soleus EMG activity until supraspinal areas can act voluntarily to appropriately modify the EMG activity. Although it is difficult to propose the exact supraspinal areas involved and the functional role of the response, this thesis provides the basis for future studies.

DANSK SAMMENFATNING

Refleksbaner mellem ekstremiteter blev beskrevet af Sherrington (1910), som demonstrerede at den krydsede extensor-refleks er til stede hos katte, og foreslog at denne refleks muligvis har en funktionel rolle. Dette arbejde lagde grunden til efterfølgende i refleksstudier hos mennesker og katte. Da det ikke er muligt at lave invasive studier på mennesker, er katte ofte brugt som model til at forstå de involverede nervebaner. Der er selvsagt store forskelle på katten og mennesket (den mest åbenlyse er firbenet vs. tobenet bevægelse). Endvidere har mennesket en forøget afhængighed af ankelens extensorer sammenlignet med katten. Selvom kattestudier viser begrænset svar fra efferente nerver i modsidige ankel extensor efter stimulation af afferente nerver fra samsidige ankel extensorer, er der en mulig forskel i afhængigheden af feedback fra begge bens ankel extensorer hos mennesket.

Formålet med denne phd-afhandling er at undersøge (i) om krydsede reflekser findes hos mennesker i siddende stilling og under gang efter elektrisk stimulation (**Studie I og III**), (ii) hvorledes de krydsede reflekser moduleres (**Study III-V**), (iii) hvilke typer nervefibre og nervebaner som kan være involveret (**Study I-III**).

Med baggrund i afhandlingen kan det konkluderes at der eksistere en kort-latens reflex mellem ekstremiteter fra den samsidige tibial nerve til den modsidige soleus muskel og at denne er (i) inhibitorisk og kan registreres i siddende stilling og under gang (ii) moduleret faseafhængigt af supraspinale områder og ændret hos patienter med apopleksi (iii) formentlig medieret af samsidige afferente nerver fra muskelsener af stor diameter.

Den foreliggende afhandling indikerer at commissurale interneuroner findes hos mennesket med input fra de samsidige anke extensor muskler eller afferente nerver fra muskelsener fra modsidige soleus muskel. Det foreslås, at stimulering af den samsidige tibialis nerve medfører en mekanisk forstyrrelse af de ipsilaterale extensorer med en initial hæmning af EMG aktiviteten i den modsidige soleus muskel indtil de supraspinale områder kan modificere EMG-aktiviteten voluntært på en hensigtsmæssig måde. Selv om det er svært at bestemme hvilke specifikke supraspinale områder der er involveret samt hvorvidt refleksen har en betydning for funktion, danner denne afhandling basis for fremtidige studier.

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ABBREVIATIONS

15cSOL/cTA	Contralateral Soleus/Tibialis Anterior muscle pre-contracted to 5–20% of the maximal voluntary contraction
30cSOL	Contralateral Soleus muscle pre-contracted to 15–30% of the maximal voluntary contraction
5-HT	5-hydroxytryptamine (serotonin)
AdExp	Additional experiments
ANOVA	Analysis of Variance
CHR	Chronic
comIN(s)	Commissural Interneuron(s)
(c)MN(s)	(Contralateral) Motor Neuron(s)
(c)SOL	(Contralateral) Soleus muscle
(c)TA	(Contralateral) Tibialis Anterior muscle
dCPN	Deep Branch of the Common Peroneal Nerve
EMG	Electromyography
EPSP(s)	Excitatory Post Synaptic Potential(s)
Flex D/HL	Nerve of the Flexor Digitorum and Hallicus Longus
GAS	Gastrocnemius
H-Reflex	Hoffmann Reflex
Hami	Hamstring nerve
HC(s)	Healthy Control(s)
(i)CPN	(Ipsilateral) Common Peroneal Nerve
IN(s)	Interneuron(s)
IPSP(s)	Inhibitory Post Synaptic Potential(s)
ISI	Interstimulus Interval
(i)SOL	(Ipsilateral) Soleus
(i)TN	(Ipsilateral) Tibial Nerve
LACI	Lacunar Infarct
M-max	Maximal peak-to-peak M-wave
MLR	Mesencephalic Locomotor Region
MpN	Medial Plantar Nerve
MVC	Maximal Voluntary Contraction
MT	Motor Threshold
n.s	Non-Significant
NA	Nor-Adrenaline
NP	Non-paretic or less affected extremity

P	Paretic or affected extremity
PACI	Partial Anterior Circulation Infarct
POCI	Posterior Circulation Infarct
PT	Perceptual Threshold
Quads	Quadriceps nerve
RMS	Root Mean Squared
SA	Sub-Acute
Sart	Sartorius Nerve
SEM	Standard Error of the Mean
SSRIs	Selective Serotonin Reuptake Inhibitors
SuN	Sural Nerve
TMS	Transcranial Magnetic Stimulation
TACI	Total Anterior Circulation Infarct

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1. INTRODUCTION

Interlimb reflex pathways were first investigated by Sherrington (1910), who demonstrated that the crossed extensor reflex was evident in the cat and suggested that it may have a functional role. This and subsequent work [Brown 1911 (cited in Zehr and Duysens 2004); Brown and Sherrington 1912; Brown 1914] formed the basis for future reflex (and interlimb reflex) studies. Since these foundation studies, interlimb reflex pathways, their afferent and supraspinal input and possible control by Central Pattern Generators have been investigated in a number animal preparations including the lamprey, cat, monkey and human (for reviews see Grillner 2003; Jankowska 2008; Jankowska and Edgley 2006; Zehr and Stein 1999). Due to the similarities between the human and other animals (although differences should also be considered; Nielsen 2003; see *section 1.5*) and the inability to perform invasive procedures in the human, much of the knowledge about interlimb reflex pathways is inferred from animal models. In the cat, a group of interneurons (INs) crossing the spinal cord [commissural INs (comINs)] have been identified. These project (directly or via INs) to contralateral motor neurons (cMNs) and likely contribute to interlimb coordination [see Jankowska (2008) for review]. The current thesis aims to investigate 1) if short-latency interlimb reflexes can be observed in the human 2) how these reflexes are modulated by supraspinal areas and 3) the likely pathways and nerve fibres involved. In particular if the comINs described from muscle afferents in the cat could also be present in the human.

1.1. Commissural interneurons (ComINs)

1.1.1. General information

Different populations of comINs have been identified in the cat and are generally defined by their input and location. Studies have been performed on the input and projections of comINs originating in lamina IV, V (Bannatyne et al. 2006; Bras et al. 1989; Matsushita 1970), VI (Bras et al. 1989; Jankowska et al. 2009), VII (Jankowska et al. 2009; also see Bannatyne et al. 2009) and VIII (Bannatyne et al. 2003; Edgley et al. 2003;

Hammar et al. 2004; Harrison et al. 1986; Jankowska and Noga, 1990; Jankowska et al. 2003, 2005a; Matsushita 1970). ComINs project to a number of areas in the contralateral spinal cord. This includes the cMNs and contralateral INs in lamina IV (one comIN in Bannatyne et al. 2006) VI–VIII (Bannatyne et al. 2003, 2006, 2009; Jankowska et al. 2009), and contralateral comINs that project ipsilaterally (Jankowska et al. 2005a). The projections of some comINs are diffuse and can have collaterals terminating on a number of contralateral lamina (Jankowska et al. 2009), located at the same level, rostrally and/or caudally (Aggelopoulos and Edgley 1995). There are four main groups of comIN located in; (1) lamina VIII with input from group II afferents only (but might have di- or trisynaptic input from reticulospinal neurons; Jankowska et al. 2005a) (2) lamina VIII with input from the reticulospinal neurons only (Jankowska et al. 2005a) (3) lamina VIII with a combination of monosynaptic input from group I, lateral vestibular nucleus (from the cerebellar fastigial nucleus) and/or reticulospinal tract (Jankowska et al. 2005a) and (4) lamina VI–VII with input from reticulospinal neurons, group Ia, Ib and II afferents (Bannatyne et al. 2009; Jankowska et al. 2009). Furthermore, there are also direct and indirect inputs from other peripheral afferents and supraspinal areas (specifically mentioned in *section 1.1.4*).

1.1.2. Afferent Input to comINs in the cat

In several studies the effect to cMNs (to a number of muscles) from stimulation of ipsilateral nerve afferents (group Ia, Ib and II; from a number of muscles) have been quantified (see below for references).

In studies where group I afferents were selectively stimulated, some studies were unable to separate the contribution of group Ia and Ib afferents (for example Holmquist 1961), observed limited contributions from group I afferents (Arya et al. 1991; Bajwa et al. 1992) and sometimes reported contradictory results (Perl 1958 vs. Holmquist 1961). Regardless, short-latency connections have been observed from group I (group Ia and Ib afferents not delineated; Jankowska and Noga 1990; Jankowska et al. 2005a, Holmquist 1961), group Ia (Baxendale and Rosenberg 1976; Harrison et al. 1986; Perl 1958, 1959) and group Ib (Baxendale and

Rosenberg 1977; Perl 1958, 1959) afferents [Note: Perl (1958) implied delineation based on stimulation intensity but stated that this conclusion should be treated with caution]. These are di, tri or poly synaptic and can be inhibitory or excitatory [see Jankowska (2008) for review].

The role of group II afferent input to comINs has been studied more extensively. Following stimulation of group II afferents, Arya et al. (1991) noted that with the spinal cord intact inhibitory post synaptic potentials (IPSPs), or no responses were recorded in the majority of cMNs. Figure 1-1 and 1-2 are a summary of the findings by Arya et al (1991) on the spinally intact cat. Figure 1-1 shows cMN responses following stimulation of specific group II muscle afferents and the percentage of cMNs with responses. The quadriceps nerve (Quads), sartorius nerve (sart), deep branch of the common peroneal (dCPN) and Flexor digitorum/hallicus longus (Flex D/HL) nerve afferents had a large percentage of cMN responses whereas afferents from the hamstring (Hami) and soleus/gastrocnemius (SOL/GAS) nerves did not. Figure 1-2 shows the cMN responses in the Hami, SOL/GAS and common TN following stimulation of any ipsilateral nerve afferent.

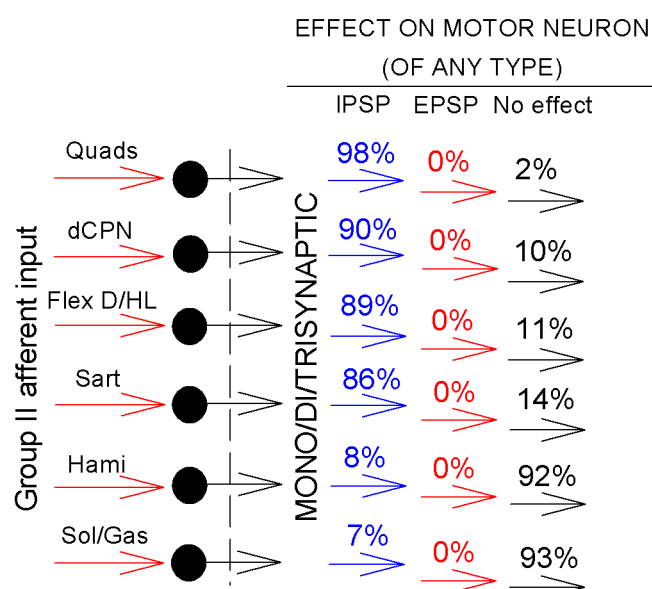


Figure 1-1

Responses in the spinally intact cat from ipsilateral group II afferents of the Quads (quadriceps nerve), dCPN (deep branch of the common peroneal nerve), Flex D/HL (flexor digitorum/hallicus longus nerve), sart (sartorius nerve), Hami (Hamstring nerve) and Sol/Gas (soleus/gastrocnemius nerve) to any cMN. The vertical dashed line indicates the centreline of the spinal cord. The black filled circles with arrows indicate interneurons with mono/di or trisynaptic input to cMNs. The blue, red and black arrows indicate the approximate percentage of cMNs with IPSPs, EPSPs, and no responses (see Arya et al. 1991) from the corresponding ipsilateral group II afferents.

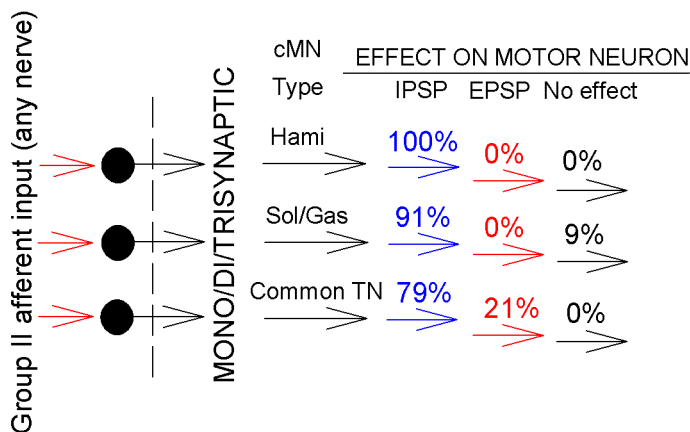


Figure 1-2

Responses in the spinally intact cat from any ipsilateral group II afferent to specific cMNs. The vertical dashed line indicates the centreline of the spinal cord. The black filled circles with arrows indicate interneurons with mono/di or trisynaptic input to cMNs. The blue, red and black arrows indicate the approximate percentage of Hami (Hamstrings), Sol/Gas (soleus/gastrocnemius) and common TN (common tibial nerve) cMNs with IPSPs, EPSPs, and no responses (see Arya et al. 1991) from the group II afferents.

Groups of comINs synapsed by group I and/or group II afferents are located in lamina VI–VIII. Additionally, two groups of INs (excitatory and inhibitory) monosynaptically excited by group II afferents have been observed in lamina IV and V of the dorsal horn. The excitatory (glutamatergic) INs were located in Lamina IV and projected predominantly ipsilaterally (to ipsilateral comINs and MNs; Bannatyne et al. 2006). The inhibitory (glycinergic) INs were located on the borders of lamina IV and lamina V and projected bilaterally (ipsilaterally - to comINs, lamina VI and VII INs and MNs; contralaterally - to lamina VII, VIII and cMNs; Bannatyne et al. 2006). ComINs, synapsed by group I and II afferents, located in lamina VI and VII had mainly excitatory actions on the contralateral lamina VI–VIII, cMNs and contralateral Ia/Ib and II INs (Jankowska et al. 2009). Of the comIN synaptic contacts in the contralateral lamina, ten were excitatory (glutamatergic) and one was inhibitory (glycinergic; Jankowska et al. 2009). Lamina VIII comIN projections to the contralateral spinal cord were either excitatory (glutamatergic) or inhibitory (glycinergic), terminated on lamina VI–VIII, contralateral INs and cMNs and had very few reported ipsilateral collaterals (Bannatyne et al. 2003, 2009; Jankowska et al. 2005b, 2009). Lamina VIII comINs with group II or reticulospinal input demonstrated different modulation by serotonin (5-hydroxytryptamine; 5-HT) and nor-adrenaline (NA). ComINs with monosynaptic input from group II afferents had more prominent responses following the administration of 5-HT and less prominent responses following administration of NA whereas comINs with reticulospinal input had more prominent responses following the administration of both 5-HT and NA (also see section 1.1.4; Hammar et al. 2004). The actions of the comINs projecting to cMNs via contralateral

lamina VI–VIII INs were facilitated by contralateral group I and II afferent stimulation indicating that these comINs project to contralateral group Ia/Ib and II INs [Cabaj et al. 2006; Jankowska et al. 2005b; also see Jankowska (2008) and Jankowska et al. (2009)].

1.1.3. Possible afferent input to comINs in humans

Due to the inability to perform invasive studies in the human, the evidence for comINs is indirect. Regardless, spinally mediated interlimb reflex pathways have been proposed from group Ia, Ib, II afferents following loading and unloading (Bachmann et al. 2008), whole body perturbations (Dietz et al. 1989), hold and release perturbations (Dietz et al. 1986) and treadmill acceleration/deceleration (Berger et al. 1984). Additionally, through stimulation of cutaneous nerves at the ankle [inferior tibial nerve: Duysens et al. 1991, sural nerve (SuN): Burke et al 1991; Delwaide et al. 1981; Duysens et al. 1991 and superficial CPN: Haridas and Zehr 2003) responses in the contralateral muscles have been observed (although these were often weak and inconsistent). The latencies of the responses in these studies were ≥ 60 –65 ms and some were delayed such that they could be supraspinally mediated [as ipsilateral reflexes in the lower limb of greater than 79 ms can be cortically mediated (Petersen et al. 1998a)]. Therefore, it is possible that some of the responses were mediated by cortical or sub-cortical areas. Although some studies have proposed muscle/tendon afferents as a source of the response (Bachmann et al. 2008; Berger et al. 1984; Dietz et al. 1986, 1989), with the methods used in these studies, distinguishing between the type (group Ia, Ib or II) of muscle afferents is difficult. Furthermore, it is also possible that the mechanical stimuli altered the position of both legs such that the observed responses may have originated from the contralateral leg.

1.1.4. Supraspinal input to comINs in the cat

Although comINs act spinally, the actions to cMNs are strongly modulated by input from supraspinal areas. One method of evaluating the effect of supraspinal input to the modulation of comINs is to completely

transect the spinal cord. In the cat, Arya et al. (1991) transected the spinal cord (see figure 1-1 and 1-2 for spinally intact cats) and noted a reversal of responses in extensor cMNs, compared to the spinally intact cat (from IPSPs to EPSPs; from any ipsilateral group II afferent). In flexor cMNs, a greater percentage of IPSPs remained following spinal transection. Figure 1-3 and 1-4 are a summary of the findings by Arya et al (1991) in the spinally transected cat. Figure 1-3 shows the cMN responses of any type following stimulation of specific group II muscle afferents, the percentage of cMNs with responses and the type of response when observed. It was stated [in Arya (1991); with reference to figure 1-3] that the reason for the limited number of IPSPs in the cMNs was that more extensor cMNs were sampled than flexor cMNs. Similarly to figure 1-1, the quads, Sart, dCPN and Flex D/HL afferents had a large percentage of cMN responses whereas the Hami and SOL/GAS nerves did not [this was also evident from group I afferent stimulation (Perl 1958; Holmquist 1961)]. Figure 1-4 shows the cMN responses in the Hami, SOL/GAS and common TN following stimulation of any ipsilateral nerve afferent. Figure 1-4 shows that the primary responses to extensor cMNs were excitatory and there was a greater percentage of IPSPs to the Hami cMNs compared to the extensor cMNs. When the spinal cord was transected unilaterally (at the dorsolateral longitudinal fasciculus), regardless of ipsilateral or contralateral transection, responses from group II afferents to contralateral extensors were inhibitory (Aggelopoulos et al. 1996). Following bilateral transection these IPSPs in the cMN changed to EPSPs. However, when 5-HT was administered (with bilateral transection) the EPSPs changed to IPSPs and following administration of a 5-HT antagonist the IPSPs changed to EPSPs. This indicates that tonic descending drive involving 5-HT neurotransmitters is a possible requirement for the generation of IPSPs from comINs, with group II afferent input, to cMNs (Aggelopoulos et al. 1996). Further, the actions of lamina VIII comINs monosynaptically excited by reticulospinal neurons had more prominent cMN responses following administration of 5-HT [and NA; Hammar et al. 2004]. Hammar et al. (2004) and Aggelopoulos et al. (1996) indicate the possible importance of pathways involving 5-HT to the modulation of comIN responses.

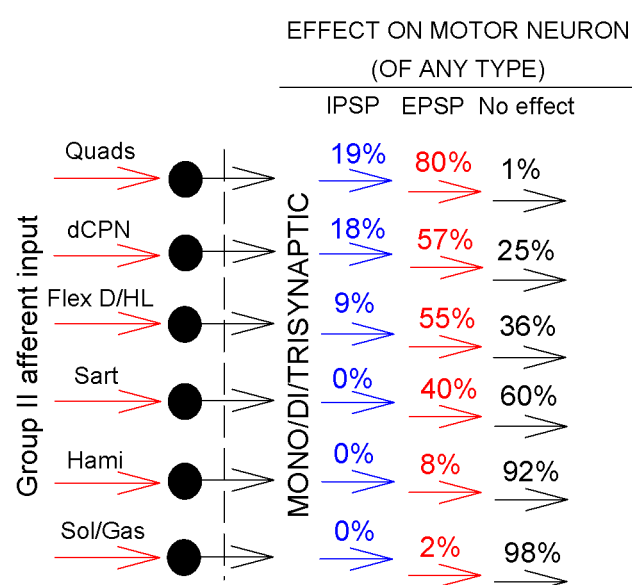


Figure 1-3

Responses in the spinally transected cat from group II afferents of the Quads (quadriceps nerve), dCPN (deep branch of the common peroneal nerve), Flex D/HL (flexor digitorum/hallicus longus nerve), sart (sartorius nerve), Hami (Hamstring nerve) and Sol/Gas (soleus/gastrocnemius nerve) to any cMN. The vertical dashed line indicates the centreline of the spinal cord. The black filled circles with arrows indicate interneurons with mono/di or trisynaptic input to cMNs. The blue, red and black arrows indicate the approximate percentage of cMNs with IPSPs, EPSPs, and no responses (see Arya et al. 1991) from the corresponding ipsilateral group II afferents.

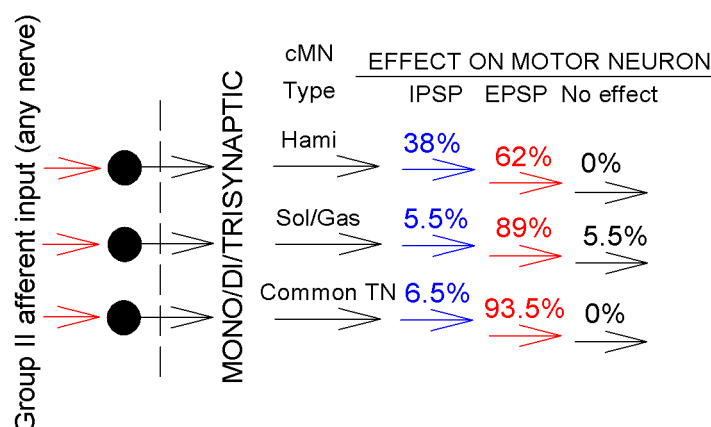


Figure 1-4

Responses in the spinally transected cat from any ipsilateral group II to specific cMNs. The vertical dashed line indicates the centreline of the spinal cord. The black filled circles with arrows indicate interneurons with mono/di or trisynaptic input to cMNs. The blue, red and black arrows indicate the approximate percentage of Hami (Hamstrings), Sol/Gas (soleus/gastrocnemius) and common TN (common tibial nerve) cMNs with IPSPs, EPSPs, and no responses (see Arya et al. 1991) from the group II afferents.

The exact supraspinal areas providing input to comINs have been investigated in the cat. There are a group of comINs in lamina VIII that receive only monosynaptic input from ipsilateral and contralateral reticulospinal tracts (Jankowska et al. 2005a). Although comINs with group II afferent input do not receive monosynaptic input from reticulospinal neurons they can receive di and/or trisynaptic input (Jankowska et al. 2005a). Another set of comINs receive input from lateral vestibular neurons, reticulospinal neurons and group I afferents (but not group II afferents; Jankowska et al. 2005a). The rubrospinal (Stecina et al. 2008), reticulospinal and vestibulospinal tracts (Jankowska et al. 2005a) have monosynaptic connections to comINs (although the corticospinal tract does not; Edgley et al. 2004). However, via the reticulospinal tract, the

ipsilateral/contralateral corticospinal tract (Edgley et al. 2004) and red nucleus (Stecina et al. 2008), ipsilateral cuneiform nucleus (Jankowska and Noga 1990) and ipsilateral mesencephalic locomotor region (MLR; Matsuyama et al. 2004) can act on comINs (or spinal INs projecting to comINs). When the MLR is stimulated directly it exhibits IPSPs and EPSPs in the ipsilateral and contralateral flexors and extensors of the hindlimb (Noga et al. 2003). Following transection superior to the mesencephalon, stimulation of the MLR results in walking, running and trotting (see Shik and Orlovsky 1976). These studies indicate that the reticular formation and MLR may have an important role in the control of comINs (reticulospinal tract) and interlimb coordination (reticulospinal tract and MLR).

1.2. Supraspinal input to reflex pathways in the human

During walking, human spinal reflexes are modulated by supraspinal areas. Previous studies on ipsilateral (and contralateral) reflex pathways have demonstrated phase modulation (for example, Andersen and Sinkjær 1999; Baken et al. 2005; Capaday and Stein 1987; Dietz et al. 1986; Duysens et al. 1990–1992; Faist et al. 1999; Sinkjær et al. 1996; Yang and Stein 1990; Zehr et al. 1998). Some of these studies (for example Andersen and Sinkjaer 1999; Capaday and Stein 1987) noted a phase modulation of responses that, by latency, could only be spinally mediated.

Although phase dependant interlimb responses from muscle afferents have been proposed during walking, the contralateral responses may not have originated from the activation of ipsilateral afferents (Berger 1984; Dietz et al. 1986; see *section 1.1.3*). Despite this, phase dependent interlimb responses following electrical stimulation of ipsilateral cutaneous afferents (a methodology which reduces the possibility of contralateral responses arising from the contralateral leg) have been observed. Through stimulation of the SuN, inferior TN and superficial CPN phase dependent responses have been evoked with onset latencies of 72–105 ms (Duysens et al. 1991 for inferior tibial nerve and SuN stimulation) and over a medium latency time window of 80–120 ms (Haridas and Zehr 2003; for superficial CPN stimulation). Duysens et al. (1991) demonstrated

that stimulation of the SuN and inferior TN evoked phase-dependent crossed spinal facilitatory responses or no responses in the cSOL and mixed responses in contralateral medial GAS. Haridas and Zehr (2003) demonstrated that stimulation of the superficial CPN evoked phase dependent middle latency (80–120 ms) contralateral responses in the tibialis anterior (TA), biceps femoris and medial GAS. These studies show that, in the human, through the gait cycle, interlimb reflex responses are probably modulated by supraspinal areas. Further evidence for the requirement of supraspinal areas to the control of spinal reflex pathways is shown by the alteration of reflexes based on the awareness of the subject (Pierrot-Deseilligny and Burke 2005: p. 4–5) and changes in responses based on the instructions to the subject (from the experimenter; Malone and Bastian 2010).

1.3. Impaired supraspinal input in the human

By removing or impairing supraspinal input, the importance of supraspinal input can be recognised.

Although investigating patients with impaired supraspinal input can demonstrate the role of supraspinal areas, due to the heterogeneity of lesions sites and symptoms (Warlow 1998), performing neurophysiological studies on these patients is difficult. Following a stroke, there are large changes to supraspinal centres (Nudo 1999, 2006). This includes a disinhibition of the non-affected hemisphere, altered reorganisation of motor maps, the degeneration of pathways due to disuse and changes in projections from supraspinal centres (see Møller et al. 2007; Nudo 1999, 2006; Thomalla et al. 2004, 2005). The lesions to supraspinal areas cause impairments to descending projections to the spinal cord. These descending projections cause impairments (such as paralysis, spasticity and sensory loss) primarily in the contralateral extremity although the extremity ipsilateral to the lesion can have impairments (for example, Khaja and Grotta 2007; Kim et al. 2003; Marque et al. 1997; Yarosh et al. 2004; Yelnik et al. 1996; Schaefer et al. 2009; Sunderland et al. 1999).

In the lower limb, the spinal mechanisms affected following a stroke include post-activation depression on the affected side (but not the unaffected side; Lamy et al. 2009), pre-synaptic inhibition (which was reduced

in acute and CHR patients on the affected side but only in acute patients on the unaffected side; Lamy et al. 2009), increased excitation of pathways from ankle flexors to knee extensors during sitting (Marque et al. 2001) and walking (Achache et al. 2010), reduced afferent mediated feedback (Mazzaro et al. 2007) and a lower threshold for stretch reflexes in the early swing phase (Nielsen et al. 1998).

1.4. Methodological considerations:

1.4.1 Recruitment of nerve afferents

Electrical stimulation is used to directly activate afferent and efferent nerve fibers bypassing the receptor-nerve interface (afferents) and stimulate muscles (efferents) without the use of descending commands. Nerve fibers have a range of properties which affect the type of nerve that is stimulated during electrical stimulation. This enables researchers to attempt to distinguish between nerve afferents. For a general review refer to Kandel et al. (2000: chpt 36).

Group Ia afferents: arise from dynamic and static nuclear bag fibers and nuclear chain fibers, are sensitive to acceleration, velocity and muscle length, have a nerve diameter of 12–20 μm and a conduction velocity of 65–68 ms^{-1} in the lower limb (Meunier et al. 1993). These are generally recruited at $< .95 \times$ motor threshold (MT; Hultborn et al. 1987) however have been recruited at stimulation intensities up to $4\text{--}5 \times$ MT (Gracies et al. 1994).

Group Ib afferents: arise from golgi tendon organs, are active during loading (and the stance phase of walking), silent during complete unloading [see Duysens et al. (2000) for review] and are generally thought to be slightly smaller and slower than group Ia afferents. These are generally recruited at stimulation intensities of $> .95 \times$ MT (Pierrot-Deseilligny et al. 1981).

Group II (muscular) afferents: arise from static nuclear bag and nuclear chain fibers, are sensitive to muscle length, have a nerve diameter of 6–12 μm and a conduction velocity of 42–48 ms^{-1} (Simonetta-Moreau et al.

1999). These are initially recruited at stimulation intensities of $> 1.2\text{--}1.5 \times \text{MT}$ (Pierrot-Deseilligny and Burke 2005: p. 293; also see Marque et al. 1996).

Group II cutaneous afferents (A β): arise from cutaneous mechanoreceptors, have nerve diameters of 6–12 μm and a conduction velocity of 45–62 ms^{-1} (Willer et al. 1978). As there is no muscle to observe an M-wave from cutaneous nerves, these are expressed as a proportion of perceptual threshold (PT). In general, stimulation intensities (below the pain threshold) from approx. $1\text{--}3.5 \times \text{PT}$ are used.

Methods such as ischemia can be used to distinguish the type of afferents involved in a response. When ischemia is administered it initially occludes the blood flow to the larger diameter (group I) afferents (Magladery et al. 1950). As there is an overlap in the diameter of group Ia and Ib afferents it is probably not possible to separate these. There is also some overlap of the largest diameter group II afferents and smallest diameter group I afferents and therefore it is possible that the transmission of group II afferents is also affected with ischemia (although it is likely that the majority of the blocked afferents are group I).

To stimulate muscle and tendon afferents single rectangular pulses of 0.5–1 ms are applied to the peripheral nerve. To stimulate cutaneous afferents one rectangular pulse does not sufficiently stimulate cutaneous nerves (Pierrot-Deseilligny and Burke 2005: p. 392) therefore a train of (generally) 3–5 pulses is used.

1.4.2. The H-reflex

The H-reflex in the SOL was initially reported by Hoffmann following surface electrical stimulation of the posterior TN at the popliteal fossa in the human (Hoffmann 1918, cited in Pierrot-Deseilligny and Burke 2005: p. 2). This has now been investigated thoroughly in the human and other animals (such as the cat) over a number of muscles.

When a peripheral nerve is stimulated the impulses to the motor efferents and sensory afferents flow in an orthodromic and antidromic direction. Larger diameter nerve fibers are recruited before smaller diameter nerve fibers. For motor efferents, this causes a reversal of the natural recruitment of motor neurons whereby

smaller diameter motor neurons are initially recruited followed by larger motor neurons (see Henneman et al. 1974). In some subjects, stimulation of the sensory afferents at low intensities will generate an H-reflex without a motor response. As the stimulation intensity increases, more sensory afferents and motor efferents are recruited. From the motor efferents, an M-wave [from orthodromic nerve flow; seen in the (electromyography (EMG) activity of the muscle] is observed, in addition to an antidromic nerve flow travelling towards the spinal cord. Initially, the sensory afferents are stimulated and the greater number of sensory afferents that are recruited the greater number of smaller motor neurons are recruited by the H-reflex (following the principles of the Henneman size principle). These physiologically recruited motor neurons do not collide with the larger antidromically stimulated motor neurons. However, as the stimulation intensity increases, smaller motor neurons are antidromically stimulated through the electrical stimulus and collide with the increasing number of orthodromically stimulated motor neurons generating the H-reflex. Once this occurs the H-reflex reduces in size until all the physiologically recruited orthodromic H-reflex motor efferents are antidromically recruited by the electrical stimulus (and the H-reflex will reduce to zero). As the stimulation intensity increases, the M-wave increases sigmoidally until the M-wave ceases to increase.

There are a number of considerations required for the use of the H-reflex. To optimally generate H-reflexes single rectangular pulses 0.5–1 ms are administered (see Panizza et al. 1989). The interstimulus interval (ISI) should be at least five seconds as if stimulated too frequently the size of the H-reflex can be reduced by post-activation depression (in the relaxed muscle; Crone and Nielsen 1989). When conditioning the H-reflex with a test reflex, an H-reflex size of 20% of the maximal peak-to-peak M-wave (M-max) should be used so that a facilitation and inhibition can be observed in the H-reflex (Crone et al. 1990). Although it was initially thought that the H-reflex was monosynaptic and only mediated by Ia afferents, more recent studies have demonstrated that only the first 0.6 ms of the H-reflex is monosynaptic (and mediated by Ia afferents; see Pierrot-Deseilligny and Burke 2005: p. 346 and 375). Following this, the effect of polysynaptic pathways (and Ib afferents) probably contribute to the size of the reflex (Marchand-Pauvert et al. 2002). This should be

considered when making conclusions based on the H-reflex.

1.5. Aims of this PhD project

Although studies report weak interlimb spinal projections from nerve afferents of the SOL/GAS complex to the cMN in the cat (Arya et al. 1991; see figure 1-1 and 1-3), due to the structural differences between the human and cat and the differing role/importance of muscles during gait there are probably differences in spinal reflex pathways between the cat and human (Nielsen 2003). These structural differences were highlighted by Nielsen (2003) who stated that humans have ‘... 1) (a) repositioning of the foramen magnum, so that the head is balanced on the spine 2) curving of the spine to keep the trunk and weight centered above the pelvis and to absorb force when the feet strike the ground 3) change of the angle between the pelvis and the spine so that the legs and spine so that the legs and spine are in line with each other 4) changes in the pelvis to support the internal organs and to make efficient attachments for the muscles that ensure the upright position 5) elongation of the lower limb 6) inward angling of the femur so that the legs are positioned more directly under the body 7) modification of the knee anatomy to allow full extension 8) (structural) modification of the feet...’. Furthermore, the role of certain muscles and the alteration of muscle synergies during gait could alter the required spinally mediated pathways. The differences in gait include ‘... (a) lean body placed above an unstable support of two long legs, the heel strike in the early part of the stance phase, the lengthening contraction of the ankle dorsiflexors in the early stance phase, the lengthening contraction of the plantarflexors throughout most of the stance phase, the controlled forward shift over the center of body mass by the propulsive power mainly of the ankle plantarflexors, and the subsequent fall of the body, which is only stopped by the initiation of the next stance phase’ (Nielsen 2003). Due to some of these differences, the ankle extensors are more important in bipedal compared to quadrupedal walking (which rely on the muscles of the hip, knee and ankle joint; Nielsen 2003). The increased reliance on the ankle extensors and differences between the cat and human could result in differences in reflex pathways.

Therefore, despite weak responses from SOL/GAS afferents in the cat it is possible that these are observed in the human. Additionally, due to the strong modulation of comINs by supraspinal areas in the cat (see *section 1.1.4*), it is possible that these are modulated by supraspinal areas in the human. Therefore, the aims of this PhD are:

1. To observe if crossed reflexes are present in sitting and walking following electrical stimulation to the ipsilateral tibial nerve [iT_N; to the contralateral soleus (cSOL)]
2. If/how crossed reflexes are modulated by supraspinal areas
3. The likely pathways and nerve fibres involved.

Five studies were performed to fulfill these aims

Study I: Short-latency crossed inhibitory responses in the human soleus muscle.

Study II: Crossed spinal soleus muscle communication demonstrated by H-reflex conditioning.

Study III: Phase modulation of the short-latency crossed spinal response in the human soleus muscle.

Study IV: Short-latency crossed spinal responses are impaired differently in sub-acute and chronic stroke patients.

Study V: Impairment of short-latency crossed spinal responses from the paretic extremity in stroke patients during gait.

Twenty eight additional experiments (**AdExp**) were performed and the results of these will be presented in this thesis to complement the various findings.

2. METHODS

2.1. Subjects

All subjects provided written informed consent to participate in the studies. Approval was given by the local ethics committees and conformed to the standards of the Declaration of Helsinki. At the time of the study all healthy subjects were free of any known physical or neurological disorders. Table 2-1 describes subject numbers, mean age \pm SEM and age range for **Study I–V**. **Table 1** (in both **Study IV, V**) describes the patient characteristics. Patients were defined as sub-acute (SA; ≤ 6 months from stroke onset) or chronic (CHR; > 6 months from stroke onset (for reasons see Brown 2006; Skilbeck et al. 1983; Swayne et al. 2008). The inclusion and exclusion criteria for the patients are described in **Study IV and V**.

Table 2-1: Subject characteristics (**Study I–V**)

Study	Number of subjects	Age (mean \pm SEM)	Minimum age	Maximum age
I	23	31 \pm 2	22	59
II	13	29 \pm 1	24	37
III	26	35 \pm 3	23	60
IV	HCS - 29	60 \pm 3	23	79
	Patients - 34	61 \pm 2	21	78
V	HCS - 21	59 \pm 4	23	79
	Patients - 21	61 \pm 2	24	76

HCS: Healthy controls

2.2. Apparatus and instrumentation

Surface electrodes (20 mm Blue sensor Ag/AgCl, AMBU A/S, Denmark) recorded the EMG activity of the SOL and TA. The electrodes were placed in accordance with Cram et al. (1998). Electrical stimulation was applied using a cathode (PALS platinum round electrode, 3.2 cm diameter) and anode {PALS platinum rectangular electrode, 7.5×10 cm, 5×9 cm or a PALS platinum round electrode, 3.2 cm diameter [for medial plantar nerve (Mpn), SuN and iCPN stimulation]}. In all experiments 1 ms rectangular pulses were

administered to the nerves. All data were sampled at a frequency of 4 kHz, high pass filtered at 10–20 Hz and low pass filtered at 1–2 kHz.

2.3. General set-up: All experiments

During testing, the electrical stimulation intensities were entered into, and controlled by a computer program. The output of this delivered random stimuli at the pre-defined stimulation intensities every 5–9 s (slightly varied between studies). The subjects were given rest breaks and were able to pause the experiment at anytime if they reported fatigue.

2.4. Establishing iTN and iCPN stimulation intensities

For all experiments the MT and M-max were established. To establish MT, the stimulus intensity was increased in 5 mA increments until an M-wave was observed. When the M-wave was observed for three trials, at the same stimulation intensity, the stimulation intensity was reduced by 1 mA. Again, three trials were observed. This process continued until no M-wave was observed. The stimulation intensity preceding this intensity was deemed the MT. Following this, the M-max was established. The stimulation intensity was increased in 5 mA increments. At each set of three trials, the preceding M-wave peak-to-peak amplitude was compared to the new M-wave peak-to-peak amplitude. Once the preceding M-wave peak-to-peak amplitude and new M-wave peak-to-peak amplitude had plateaued for three trials, the electrical stimulus was decreased to the previous stimulation intensity and labelled the M-max. From the peak-to-peak M-wave at M-max, 85% M-max (**Study II–V**), 35% M-max {**Study III and Study V** [healthy controls (HCs) only]} were calculated and established (or a range of stimulus intensities were collected). The peak-to-peak M-wave of the stimulated muscle was monitored online to ensure consistency throughout testing.

2.5. Sitting experiments (*STUDY I, II, IV and AdExp*)

In all experiments the hip and knee were positioned at an angle of 100° and the ankle at 110° (figure 2-1).

The right and left feet were positioned on two separate footplates aligned parallel to each other (figure 2-1).

All stimulating and recording electrodes were attached in standing.

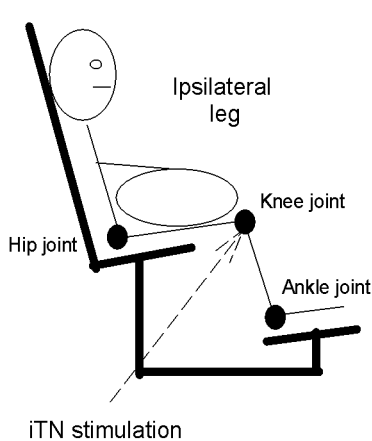


Figure 2-1

Experimental set up for sitting protocol



2.5.1. Muscle pre-contraction

The cSOL (**Study I, II, IV**)/cTA (**AdExp**) were pre-contracted to 5–20% (pre-contraction level slightly varied between studies; 15cSOL/15cTA) and/or 15–30% (30cSOL) of the maximum voluntary contraction (MVC). For the patients in **Study IV** the SOL opposite to the stimulated TN was pre-contracted to 15cSOL of the paretic (P) SOL. Initially, the subjects were asked to perform an MVC of the cSOL [**Study I, II, IV (HCs) and AdExp**], P SOL [**Study IV (patients)**] or cTA (**AdExp**). For the pre-contracted SOL MVC, the subjects were instructed to push down and perform a maximal isometric contraction of their pre-contracted SOL so that the resulting force was due to the contraction of the plantarflexors. For the cTA MVC, the subjects were instructed to ‘pull up’ the foot at the ankle and perform a maximal isometric contraction of the cTA so the resulting force was due to the contraction of the dorsiflexors. While performing the MVC

measurements the subjects were asked to minimise contraction of the proximal and antagonist muscles (monitored by observation). If this occurred, the trial was discarded and repeated until a total of three successful MVC measurements were recorded (with 1 minute rest between measures). The EMG activity of the pre-contracted SOL and cTA were displayed on a screen in front of the subjects. During the experiments, arrows on a bar indicated the upper and lower limits (percentage of the MVC to be contracted to). The instructions to the subjects were to maintain the required EMG, between the two arrows, at all times.

2.5.2. iTN and iCPN stimulation

For TN stimulation during sitting (**Study I, II, IV and AdExp**), the cathode was located in the popliteal fossa and the anode was located on the anterior aspect of the leg at the level of the patella. The cathode was pressed into the skin with a ball affixed to the distal thigh to optimise the observed M-wave and minimise the stimulation artifact in the stimulated SOL EMG trace. If this was not observed, the cathode and ball were removed and reattached to a different site in the popliteal fossa until an optimal position had been located. For iCPN stimulation (**AdExp**), the stimulating electrodes were placed at the level of the fibula head (with the cathode superior to the anode). The location of the stimulating electrodes varied slightly between subjects. The electrodes were positioned so that at $2 \times \text{MT}$ of the iTA following stimulation of the iCPN, there was minimal contraction of the peroneal muscles. At higher stimulation intensities, co-contraction of the TA and peroneal muscles was unavoidable. Once MT of the TA had been established, the stimulation intensity was increased to $2 \times \text{MT}$. The peroneal muscles were palpated and if these were activated, the electrodes were removed and placed at another site around the fibula head. This continued until no/minimal co-contraction of the peroneal muscles was observed. For some subjects, it was not possible to completely isolate the TA at $2 \times \text{MT}$.

2.5.3. SuN and MpN stimulation (STUDY I)

For SuN and MpN stimulation, the stimulating electrodes were attached posterior and inferior to the medial malleolus for MpN stimulation and posterior and inferior to the lateral malleolus in the notch between the lateral malleolus and calcaneal tendon for SuN stimulation. Three consecutive stimuli; 3 shocks, 3 ms interval, 1 ms duration (see Nielsen et al. 1997) were applied at an intensity of 5 mA. The subjects were asked to describe the sensation. If the desired sensation was not reported, the electrodes were moved until this was felt. The desired sensation for MpN stimulation was a triangularly spreading sensation toward the first and second metatarsal on the plantar side of the foot and for SuN stimulation was a sensation on the lateral side of the foot toward the fifth metatarsal. Once a suitable location had been located, the stimulation intensity was lowered so the subjects reported feeling no stimulus. From this level, the stimulation intensity was increased by 0.1–0.2 mA increments for three trials until the subjects reported the feeling of an electrical stimulus. Once this had occurred, the stimulation intensity was increased by 0.5–1 mA and then lowered by 0.1–0.2 mA until the subject ceased to feel the electrical stimulus for three trials. The stimulation intensity between which the subject began to feel and ceased to feel the electrical stimulus was deemed PT. For testing, 60 stimuli were delivered every 5–7 seconds at stimulation intensities of $1-3 \times \text{PT}$ while the cSOL was contracted to 15cSOL.

2.5.4. Application of ischemia (STUDY I)

Prior to ischemia, M-max, 75% M-max, H-max and control reflex were established following stimulation of the iTN. The 15cSOL contraction level was used for the experiment and was maintained during iSOL H-reflex collection. Initially, 20 H-reflexes of the iSOL, following stimulation to the iTN were collected. This was followed by 40–60 stimuli at 75% M-max delivered every 5–7 seconds. Ischemia was applied to the stimulated leg at the distal thigh superior to the knee joint (and stimulation site). The blood pressure cuff was inflated to 200–220 mmHg. Following cuff inflation, the H-reflex was assessed at 5, 10 and 15 minutes.

After 15 minutes the H-reflex was assessed every 5–7 s. When the H-reflex was depressed to 25% of its pre-ischemia value (approximately 20–22 minutes), 40–60 electrical stimuli were delivered at 75% M-max with the cSOL contracted to 15cSOL. The H-reflex size of 25% of the pre-ischemic value was chosen as Uysal et al. (2009) demonstrated that this level of ischemia blocks approximately 50% of group Ia afferents without affecting the group II afferents. The peak-to-peak M-wave was monitored online to ensure this did not decrease. A reduction of the peak-to-peak M-wave would indicate that the smaller diameter α -MNs (and consequently smaller diameter sensory afferents) were blocked. Once the peak-to-peak M-wave began to decrease, electrical stimulation ceased and the cuff was slowly deflated. Fifteen minutes following ischemia, 20 H-reflexes were assessed to ensure the peak-to-peak H-reflex had returned to its baseline level. Once this had occurred, 60 electrical stimuli were applied at 75% M-max with the cSOL contracted to 15cSOL. The data were analysed offline to ensure there was no reduction in the peak-to-peak M-wave amplitude during ischemia. If the M-wave had reduced, the subsequent trials were removed from the analysis.

2.5.5. Conditioning of the cSOL H-reflex (STUDY II)

For the second part of **Study II**, the iTN was stimulated at 85% M-max while test H-reflexes were delivered to the cSOL following stimulation of the contralateral TN. From stimulation of the contralateral TN, the MT, M-max, H-max and an H-reflex size of approximately 20% M-max of the cSOL were established. A test H-reflex size of approximately 20% M-max was conditioned (used in Petersen et al. 1998b) as the H-reflex is sensitive to inhibition and facilitation at this level (Crone et al. 1990). The test cSOL H-reflex was elicited at -6–100 ms following iTN stimulation. In addition, a control reflex, with no conditioning stimulus, was administered. The different pairings of stimuli (including the control reflex) were administered randomly every 5–9 seconds totalling 510 pairs of stimuli. The control stimuli were monitored online to ensure consistency throughout testing.

2.6. Walking experiments (STUDY III and V)

2.6.1. General protocol

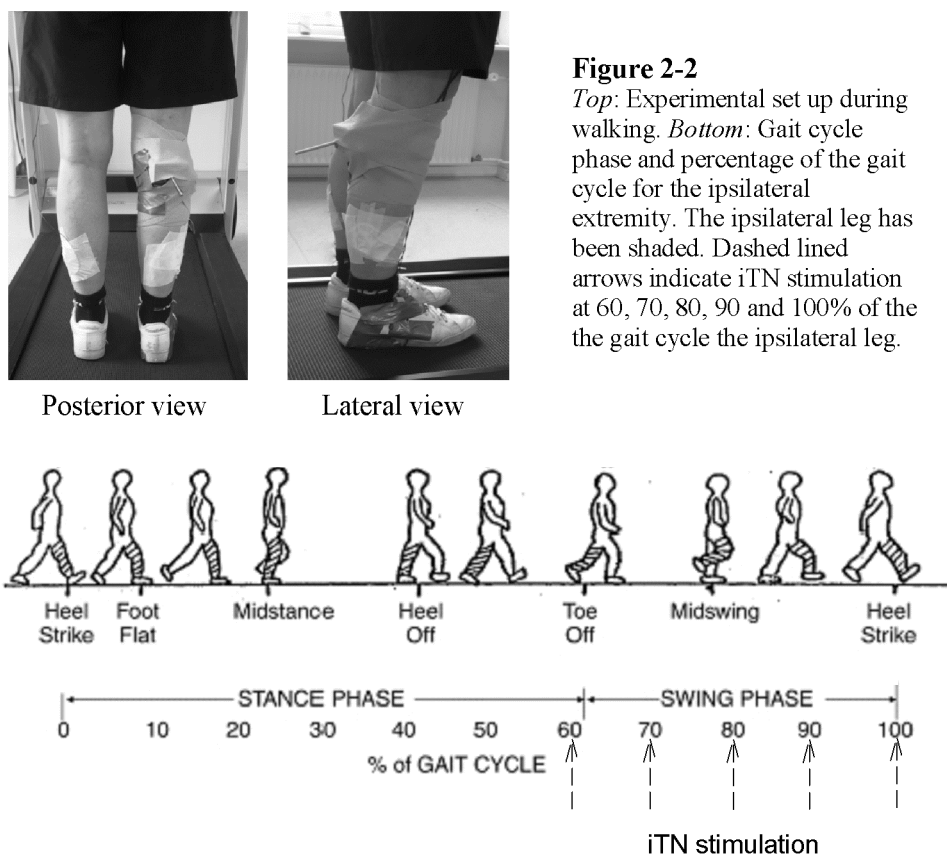
The subjects walked on a treadmill at 3.5 kmh^{-1} (**Study III**) or at a comfortable speed (**Study V**; HC walking speed: 2.9 kmh^{-1} ; patient walking speed: 1.5 kmh^{-1}).

All stimulating and recording electrodes were attached in standing. The heel contact was signalled with a force sensor attached to the heel of the stimulated leg. Prior to testing, the subjects walked for 5–10 minutes to become accustomed to the treadmill and walking speed. Following this, 10–30 steps were recorded to establish the non-stimulated walking profile of the subject. From the walking profile, 60% (Note: some subjects displayed no EMG activity in the non-stimulated SOL at 60% of the gait cycle. For this reason, these subjects were stimulated up to 65% of the gait cycle), 70%, 80%, 90% and 100% of the gait cycle [**Study III** (iTN experiments)] and 90% of the gait cycle (**Study III** for MpN and SuN experiments; **Study V**) were calculated. The gait cycle was defined as one ipsilateral heel contact (corresponding to 0% of the gait cycle) to the next ipsilateral heel contact (corresponding to 100% of the gait cycle). Figure 2-2 (bottom) shows the gait cycle as defined by the ipsilateral leg and the location of stimulation. Pre-defined stimuli were delivered every 3–5 steps. For **Study V**, all subjects (patients and healthy controls) held the handrail on the treadmill for support. All subjects took rest breaks as required [**Study III**: between protocols; **Study V** (HCs): 1–3 rest breaks; **Study V** (patients): 3–5 rest breaks]. For the rest breaks, the subjects were standing (holding onto rails) or sitting down. If subjects began to vary from their initial stride time ($\pm 50 \text{ ms}$) they were asked to increase or decrease their stride time. The peak-to-peak M-wave of the stimulated muscle was monitored online to ensure consistency throughout testing. Data were analysed offline.

2.6.2. TN stimulation during walking

For TN stimulation the cathode was pressed into the skin with a rod affixed to the distal thigh. To affix the rod and minimise the movement artifact in the recording electrodes the lower legs were wrapped in a medical

bandage, applied with enough pressure to keep the rod in place without restricting the movement of the leg [see figure 2-2 (top)]. For all subjects MT and M-max were established. This was done individually for each tested phase of the gait cycle (**Study III**) and the P and non-paretic (NP) extremity (**Study V**). To establish these, stimuli were delivered every 3–5 steps. The stimulation intensities were established as in ‘section 2.4’. During testing, the TN was stimulated at MT, 35% M-max and 85% M-max [**Study III**; **Study V** (HCs)] and 85% M-max only [**Study V** (patients)]. In **Study III**, seven subjects were stimulated at different stimulation intensities ranging from no stimulus to M-max. For all walking experiments, a ‘no stimulation’ (control) condition was included.



2.6.3. MpN and SuN stimulation (STUDY III)

All subjects performed both experiments. For **Study III**, the method for establishing the PT was the same as ‘Section 2.5.3’. For testing, the subjects were stimulated at $1 \times$, $2 \times$ and $3 \times$ PT (including a ‘no stimulation’ (control) condition) every 3–5 steps at 90% of the gait cycle for a total of 45–60 recordings per stimulation intensity.

2.7. Measurements recorded

2.7.1. Magnitude using the minimum value (STUDY I)

For the iTN experiments in **Study I**, the cSOL inhibitory response was quantified as the minimum value within a 30–60 ms time window following iTN stimulation. The minimum value was expressed as a percentage of the RMS of the cSOL background EMG, recorded in the 90 ms preceding the electrical stimulus to the iTN. When an inhibition was observed the inhibition magnitude, onset, duration and time of minimum value were recorded. For MpN and SuN experiments in **Study I**, the cSOL response was quantified as a minimum value within a 38–68 ms time window following iTN stimulation. A delay of 8 ms was added as the MpN and SuN were stimulated at the ankle and the iTN was stimulated at the popliteal fossa. Assuming the velocity of the low threshold ($A\beta$) cutaneous afferents is approximately $45\text{--}62\text{ ms}^{-1}$ (Willer et al. 1978), with a tibial leg length of approximately 0.4 m, a delay of 8 ms would be expected compared to electrical stimulation applied at the popliteal fossa.

2.7.2. Magnitude using root mean squared (RMS; STUDY II–V, AdExp)

In **Study II–V and AdExp**, the analysis was changed from the minimum value as a percentage of the baseline RMS (**Study I**) to an RMS over a specific time window [RMS response window was 40–55 ms following iTN stimulation (for subjects younger than 50 y/o) and 45–60 ms (for subjects older than 50 y/o)] as a percentage of the baseline RMS (**Study II, IV and AdExp**) or control step RMS (**Study III and V**). For

MpN and SuN stimulation during walking (**Study III**), 8 ms was added to the time window (see *section 2.7.1*).

Different time windows were chosen for older and younger subjects due to the large discrepancy in the age of the subjects in **Study IV and V** (see table 2-1). Assessing the older and younger subjects over the same time period seemed inappropriate as nerve conduction velocity has a negative correlation with age (for example, Bouche et al. 1993; Buchthal and Rosenfalck 1966; Maglietta 1969). Therefore, the age of the subjects *vs.* the time of the minimum value (in sitting) was considered. From the data during sitting (**Study I, II, IV and AdExp**), when assessing the time of the minimum value *vs.* the age of the subject, 45/49 subjects showed a time of minimum within the time windows (Note: for subjects that appeared in > 1 study, the results from the most recent testing session were used).

2.7.3. Reasons for the change in analysis.

Following **Study I**, the analysis of data was changed. For some subjects, no response or facilitatory responses were observed. Therefore, assessing these subjects with a minimum value was inappropriate. Despite this, the analysis used in **Study I** was appropriate given that, at the time of writing, there was less information with regards to the nature of the response. By **Study II**, more pilot and experimental data had been collected and the analysis of the response was refined. Using a minimum value could generate errors and overestimate the size of the reflex, as a minimum value is a single point and could occur by chance alone (also see **Study II**). With this in mind, using an RMS was a better indicator of the magnitude of the response.

2.8. Stroke classification and clinical measurements

The lesion site of the patients was classified using the Bamford classification (see Bamford et al. 1991). When able, the site of stroke was confirmed using a computed tomography scan performed within 24 hours of the initial event. The clinical measurements were the 10 meter walk test, Fugl-Meyer Lower limb

assessment (Fugl-Meyer et al. 1975), Ashworth scale (Ashworth 1964), Babinski sign (present/absent) and clonus (present/absent).

2.9. Statistical analysis

Repeated-measures ANOVA (response variable: magnitude of response for each subject, within factor variable: stimulus intensity) were performed in **Study I** [for iTN stimulation (including ischemia experiments)], **Study III** (at 60%, 70%, 80%, 90% and 100% of the gait cycle), **Study IV** [for P–NP and NP–P (also for SA and CHR separation) and HCs], **Study V** (for HCs) and the **AdExp** (iTN to cTA, iCPN to cSOL, iCPN to cTA). For **Study II**, a repeated measures ANOVA was performed (response variable: test H-reflex as a percentage of the control H-reflex, within factor variable: ISI). One-way ANOVA were performed in **Study III** for each stimulation intensity (response variable: magnitude of the response, factor variables: 60%, 70%, 80%, 90% and 100% of the gait cycle) and **Study I and III** [response variable: magnitude of response, factor variable: SuN and MpN (at the various intensities) and iTN stimulation (75% M-max for sitting and 85% M-max for walking)]. For **Study V** the P–NP vs. NP–P extremities were compared with paired t-tests and the P–NP/NP–P vs. HCs were compared with an unpaired t-test. When mass significant differences were revealed Fishers LSD tests were performed post-hoc to establish the location of the significant differences. In **Study IV**, a Levene test revealed variability violations when comparing SA (P–NP), SA (NP–P), CHR (P–NP), CHR (NP–P) and HCs at 85% M-max. The Levene test was used to assess the mass variance heterogeneity (heteroscedasticity) between groups [Note: Due to the heteroscedasticity in sitting (**Study IV**), the Levene test was also performed for comparative purposes in walking (**Study V**) however there was no mass significant effect]. Levene tests were performed post-hoc to determine significant differences in variability between the individual groups (for 85% M-max; **Study IV**). Due to the heteroscedasticity at 85% M-max, a Welch ANOVA was administered and Welch t-tests were performed post-hoc to determine the location of the differences between groups (**Study IV**).

For **Study IV and V**, linear regression analysis were performed on the RMS of the response (as a percentage of the baseline RMS) vs. 10 meter walk time, Fugl-Meyer lower limb score and Ashworth scale for the P–NP, NP–P for all patients and separately for SA (P–NP), SA (NP–P), CHR (P–NP) and CHR (NP–P) with the iTN stimulated at 85% M-max.

3. RESULTS

3.1. Responses in the cSOL following iTN stimulation.

In healthy subjects during sitting a short-latency inhibitory response was observed in the pre-contracted cSOL following stimulation of the iTN. The magnitude of the inhibition became more prominent as the stimulus intensity increased. The average onset, duration and time of minimum ranged from 37.1–40.8 ms, 19.6–26.9 ms and 46.4–50.5 ms, respectively (see table 3-1). There were no significant differences between the onset, duration or time of minimum of response at the same iTN stimulation intensity comparing 15cSOL vs. 30cSOL ($P > .05$) or over stimulus intensities for 15cSOL and 30cSOL ($P > .05$). There were no significant differences in the magnitude of the response comparing 15cSOL vs. 30cSOL for the same stimulus intensities ($P > .05$). Table 3-1 shows the average magnitude, onset, duration and time of minimum of the responses at the contraction level of 15cSOL and 30cSOL for all subjects. Figure 3-1(A–E) shows raw cSOL traces from an individual subject at the 15cSOL contraction level stimulated at 0, 25, 50, 75 and 100% M-max of the iSOL. Figure 3-2(A and B) shows the mean \pm SEM for all subjects for 15cSOL (Fig. 3-2A) and 30cSOL (Fig. 3-2B) with the iTN stimulated at 0, 25, 50, 75 and 100% M-max of the iSOL. Figure 3-1, 3-2 and table 3-1 show an inhibitory response in the cSOL becoming more prominent as the stimulus intensity increases.

Table 3-1: Magnitude (from the minimum value), onset, duration and time of minimum of the inhibition.

M-max %	Magnitude of depression (%)		Onset of depression (ms)		Time of minimum value (ms)		Duration of depression (ms)	
	15cSOL	30cSOL	15cSOL	30cSOL	15cSOL	30cSOL	15cSOL	30cSOL
0	87.2 \pm 2.8	86.8 \pm 2.7	-	-	-	-	-	-
25	76.9 \pm 3.2	77.1 \pm 4.2	39.5 \pm 1.5	40.8 \pm 1.8	50.5 \pm 1.3	49.7 \pm 1.3	23.7 \pm 4.4	19.6 \pm 2.8
50	76.1 \pm 2.9	76.7 \pm 1.6	37.6 \pm 1.2	38 \pm 1.1	47.3 \pm 1.8	46.4 \pm 2.1	26.9 \pm 4.9	24.2 \pm 2.8
75	72.1 \pm 2.9	72.8 \pm 2.6	38.5 \pm 1.4	40 \pm 1.4	48.6 \pm 1.3	49.9 \pm 1.5	24.1 \pm 2.4	26.6 \pm 4.9
100	68.7 \pm 3.3	69.2 \pm 2.2	40.4 \pm 1.7	37.1 \pm 1.1	50.4 \pm 1.7	46.8 \pm 1.1	24.8 \pm 2.4	26.3 \pm 3

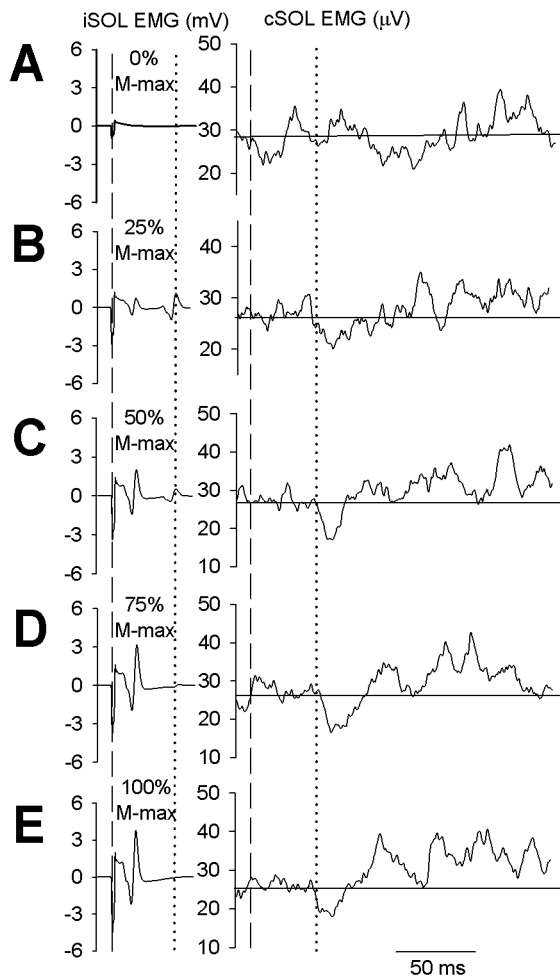


Figure 3-1

Raw electromyographic (EMG) traces of the ipsilateral and contralateral soleus (iSOL and cSOL) following different ipsilateral tibial nerve (iTAN) intensities ($n=1$). The percentage of M-max for the iSOL and the corresponding cSOL trace are shown. *A*: 0% M-max; *B*: 25% M-max; *C*: 50% M-max; *D*: 75% M-max; *E*: 100% M-max. The vertical dashed lines represent the iTAN stimulus onset, the vertical dotted lines represent 40 ms post stimulus onset and the horizontal dashed line represents the approx. background EMG. Each trace is the average of 30 trials.

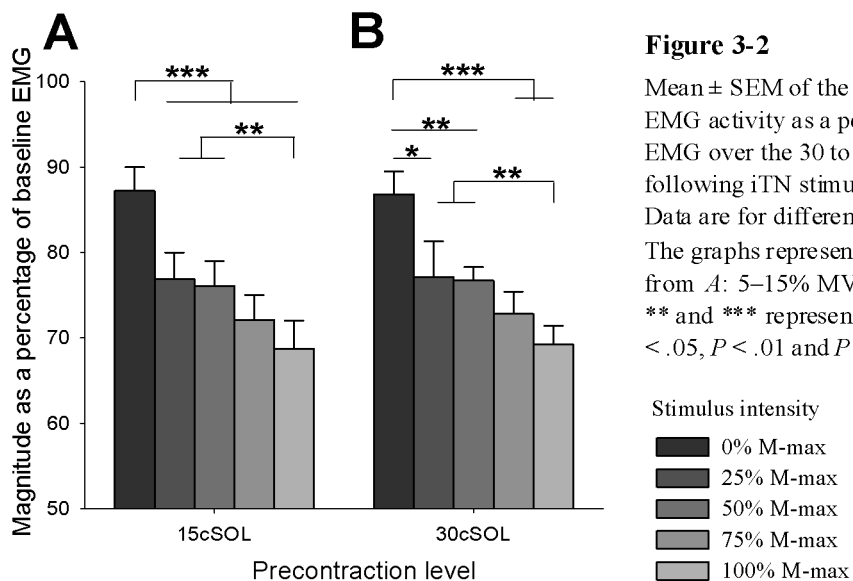
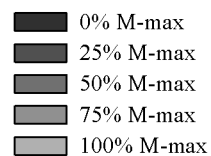


Figure 3-2

Mean \pm SEM of the minimum value of the cSOL EMG activity as a percentage of the background EMG over the 30 to 60 ms time window following iTAN stimulation across all subjects. Data are for different iTAN stimulation intensities. The graphs represent a cSOL contraction level from *A*: 5–15% MVC and *B*: 15–30% MVC. *, **, and *** represent significant differences to $P < .05$, $P < .01$ and $P < .001$.

Stimulus intensity



In healthy subjects during walking, a short-latency inhibitory response was observed in the cSOL towards the swing to stance transition phase of the ipsilateral leg. The magnitude of the inhibition became more prominent as the stimulus intensity increased at 80, 90 and 100% of the gait cycle ($P \leq .01$; see *section 3.5.1*). For all subjects over all phases and stimulation intensities the average onset of the response was 39.5 ± 0.4 ms and the duration was 24.7 ± 1.1 ms. There were no significant differences between the onset and/or duration of the response at any phase or stimulation intensity ($P > .05$). Figure 3-3 shows raw cSOL traces from an individual subject stimulated at MT, 35% M-max and 85% M-max at 90% of the gait cycle. Figure 3-4 shows the mean \pm SEM for all subjects stimulated at 85% M-max for 90% of the gait cycle. Figure 3-3 and 3-4 show an inhibitory response in the cSOL that becomes more prominent as the stimulation intensity increases.

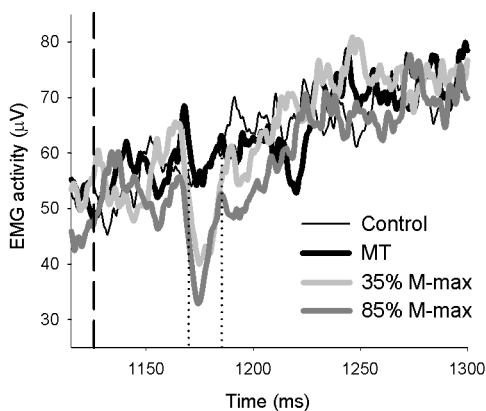


Figure 3-3

Raw cSOL EMG traces with an average of 40 traces over a 150 ms time window following iTN stimulation at MT, 35% M-max and 85% M-max for 90% of the gait cycle of the ipsilateral leg ($n = 1$). The thin black line represents the control step, the thick black line represents stimulation at MT, the thick light grey line represents stimulation at 35% M-max and the thick dark grey line represents stimulation at 85% M-max. The vertical long dashed line represents stimulation of the iTN, the vertical dotted lines represent the analysis window, 45–60 ms following iTN stimulation (as this subject was 60 y/o).

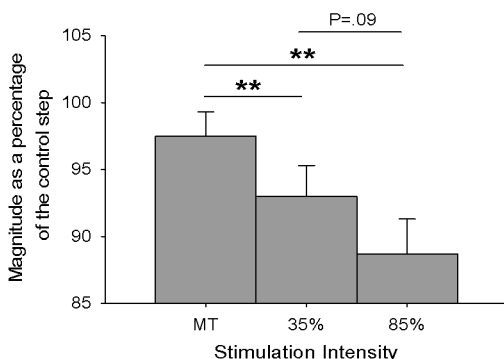


Figure 3-4

Mean \pm SEM of the magnitude of the cSOL response as a percentage of the control step (for the age appropriate analysis windows) across all subjects ($n = 16$) at 90% of the gait cycle following stimulation at MT, 35% M-max and 85% M-max. ** represents significant differences to $P < .01$. Near significant differences are indicated.

3.2. Potential afferents involved in the crossed spinal response

Due to the onset latency of the inhibition (see *section 3.1*), it is likely that the response is mediated by ipsilateral group I and/or group II afferents and not ipsilateral A δ or C afferents.

Group I afferents: During ischemia (which selectively blocks group I afferents) the time of the minimum of the response was delayed compared to pre and post ischemia ($P < .05$). The magnitude, onset and duration of the response were not significantly altered pre, during and post ischemia ($P > .05$). Ischemia eliminated the response in one subject. The average onset of the inhibition occurred at intensities below MT in three subjects.

Group II muscle afferents: The average onset of the cSOL inhibition occurred at $1.2 \times \text{PT}$. The average onset of the inhibition occurred at intensities above MT in eight subjects. As the stimulus intensity increased, the inhibition became more prominent (which may indicate the additional recruitment of group II and/or group I afferents).

Group II cutaneous afferents: Stimulation of the ipsilateral MpN and SuN at the ankle showed no short-latency responses during sitting or walking at any stimulation intensity ($1-3 \times \text{PT}$). The magnitude of the response was significantly less prominent when compared to stimulation of the iTN at 75% (sitting) and 85% M-max (walking) (post-hoc tests: $P < .001-P < .05$).

iCPN nerve afferents: At higher stimulation intensities of the iTN, it is possible that nerve afferents of the iCPN were activated (causing the short-latency response). Stimulation of the iCPN to the pre-contracted cSOL demonstrated no short-latency inhibitory responses at any stimulus intensity (when assessed over the same time period as the iTN to cSOL). When compared to stimulation of the iTN to cSOL at 85% M-max, the responses were significantly less prominent ($P < .001-P < .01$). Figure 3-5(A) shows representative raw EMG data of the cSOL following stimulation of the iCPN at MT and 85% M-max ($n = 1$). Figure 3-5(B) shows the mean \pm SEM of the magnitude of the response for all subjects at each tested stimulation intensity. As a comparison the mean \pm SEM for the iTN to cSOL stimulated 85% M-max is presented (with significant

differences and level of significance compared to 85% M-max). Figure 3-5 shows no short-latency crossed inhibition from the iCPN to cSOL [when assessed over the same time period as the iTN to cSOL (stimulated at 85% M-max)].

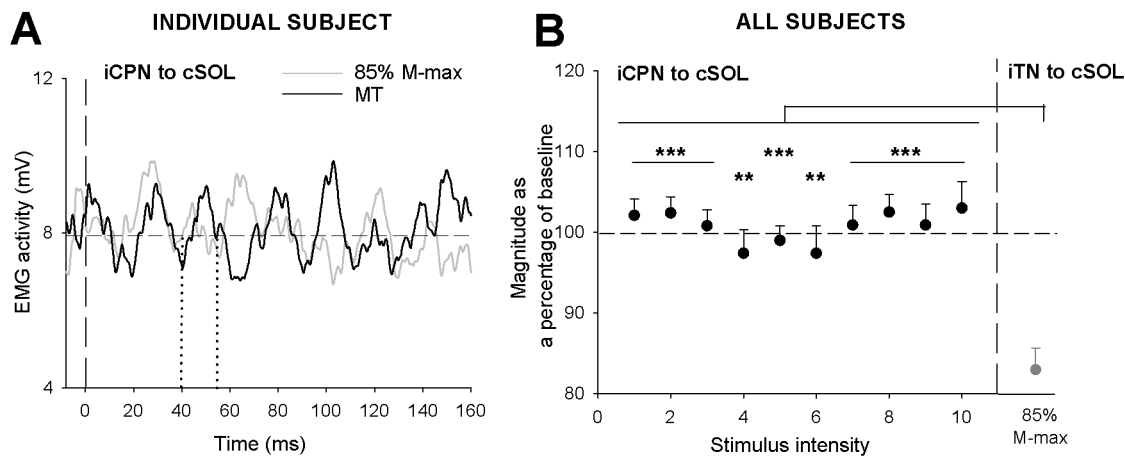


Figure 3-5

A: cSOL EMG for an individual subject following stimulation of the iCPN at MT (black line) and 85% M-max (grey line). The vertical dashed line represents stimulation of the iCPN, the vertical dotted lines represent the analysis window. Each trace is the average of 35 trials. B: Mean \pm SEM of all subjects at each tested stimulation intensity for the iCPN to cSOL. As a comparison the mean \pm SEM for the iTN (to cSOL) stimulated at 85% M-max (analysed over the same time window) is shown. *** and ** represent significant differences [compared to iTN to cSOL (at 85% M-max)] to $P < .001$ and $P < .01$, respectively.

3.3. Responses from the iCPN to cTA and iTN to cTA (AdExp)

There were no short-latency crossed responses (when analyzed over the same analysis window as the iTN to cSOL) to the cTA following stimulation of the iTN and iCPN (during sitting) and these were significantly different to the inhibitory responses of the iTN to the cSOL (stimulated at 85% M-max; $P < .01$ – $P < .001$).

Figure 3-6(A and B) shows representative raw EMG data of the cTA following stimulation of (A) iCPN and (B) iTN at MT and 85% M-max. Figure 3-6(C and D) shows the mean \pm SEM of the magnitude of the response for all subjects at each tested stimulation intensity. As a comparison the mean \pm SEM for the iTN to cSOL stimulated 85% M-max is presented (with significant differences and level of significance compared to

85% M-max). Figure 3-6 shows no short-latency crossed inhibition from the iCPN–cTA or iTN–cTA in the same defined time windows as from the iTN–cSOL.

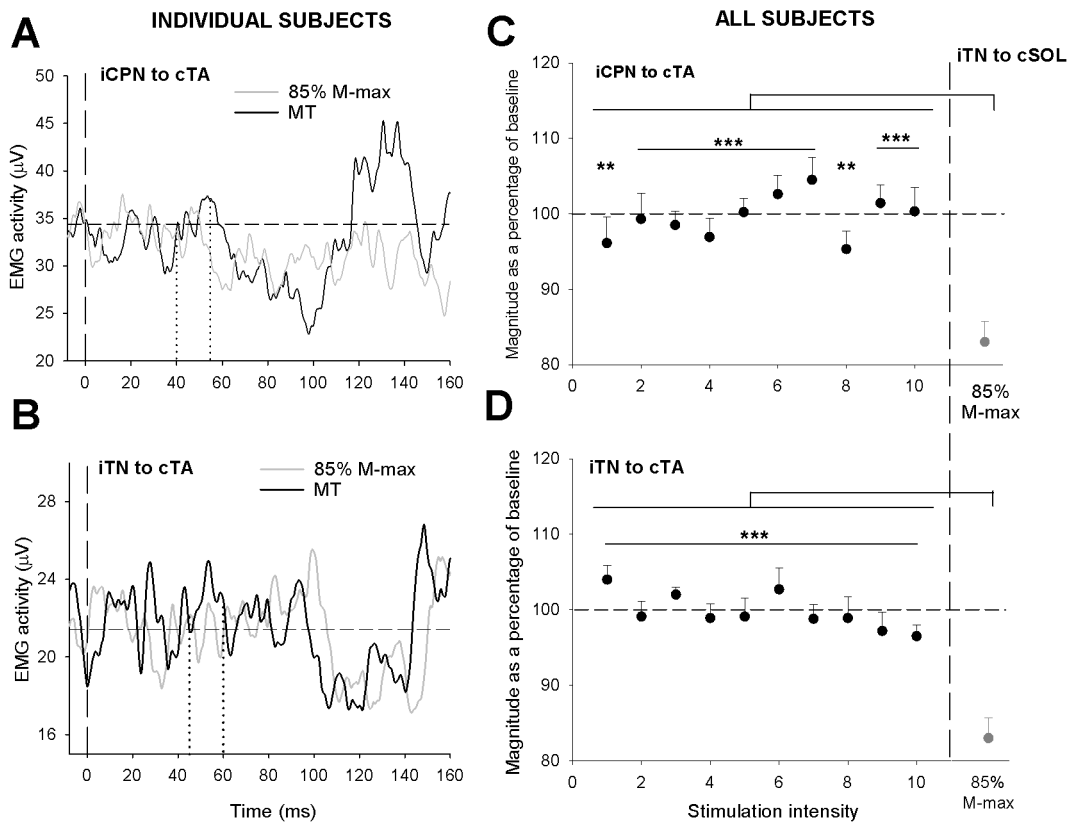


Figure 3-6

A and B: cTAEMG for individual subjects following stimulation of (A) iCPN and (B) iTN at MT (grey line) and 85% M-max (black line). The vertical dashed line represents stimulation of the iCPN, the vertical dotted lines represent the age appropriate analysis window. C and D: Mean \pm SEM of all subjects for each stimulation intensity tested for the iCPN and iTN to the cTA. As a comparison the mean \pm SEM for the iTN to cSOL (stimulated at 85% M-max; analysed over the same analysis window) is shown. *** and ** represent significant differences [compared to iTN to cSOL (at 85% M-max)] to $P < .001$ and $P < .01$, respectively.

3.4. H-reflex conditioning

The repeated measures ANOVA revealed a significant inhibition ($P < .001$) in the cSOL H-reflex, compared to the control (no) stimulus, when conditioning stimuli were delivered at ISIs of 3–33 ms before the test stimuli (post-hoc tests: $P < .05$ – $P < .001$). When comparing conditioning of the H-reflex and the pre-contracted cSOL, paired t-tests demonstrated no significant difference in the magnitude of the response or

time of the minimum value ($P > .05$). Figure 3-7 displays the magnitude of the conditioned H-reflex as a percentage of the control reflex (mean \pm SEM) at each ISI time point for all subjects. As the average H-reflex onset was 31.4 ms, the ISIs were standardized to this value. The graph shows a significant inhibition (significance levels not indicated in the diagram), an average minimum value occurring at 46.4 ms (ISI of 15 ms) and an average magnitude (at an ISI of 15 ms) of $84.6 \pm 2.6\%$ (of the control H-reflex).

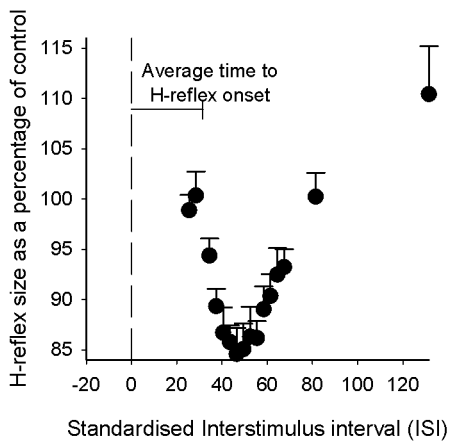


Figure 3-7

Mean \pm SEM of the magnitude of the conditioned reflex as a percentage of the control reflex at each ISI time point (black filled circles) for all subjects ($n = 13$). As the average H-reflex onset was 31.4 ms, the ISI was standardised to this value.

3.5. Supraspinal modulation of the response

3.5.1. Phase-dependence during walking

The magnitude of the responses were significantly different over the phase of the gait cycle at iTN stimulation intensities of 85% M-max ($P < .01$) and 35% M-max ($P = .01$). At these stimulation intensities the inhibitory response became more prominent towards the swing to stance transition (100% of the gait cycle) of the ipsilateral leg. Figure 3-8(A–F) shows raw cSOL EMG traces for one subject following stimulation of the iTN at 85% M-max. Figure 3-8(A) displays a normal cSOL EMG trace over the entire gait cycle and the location of iTN stimulation for this subject. Figure 3-8(B–F) displays (with truncated axis) the cSOL EMG following iTN stimulation. Figure 3-9 shows the mean \pm SEM of the response magnitude as a percentage of the control step stimulated at 85% M-max (Fig. 3-9) for 60%, 70%, 80%, 90% and 100% of the

gait cycle. Figure 3-8 and 3-9 show that the inhibition becomes more prominent towards the swing to stance transition (100% of the gait cycle) of the ipsilateral leg (at 85% M-max).

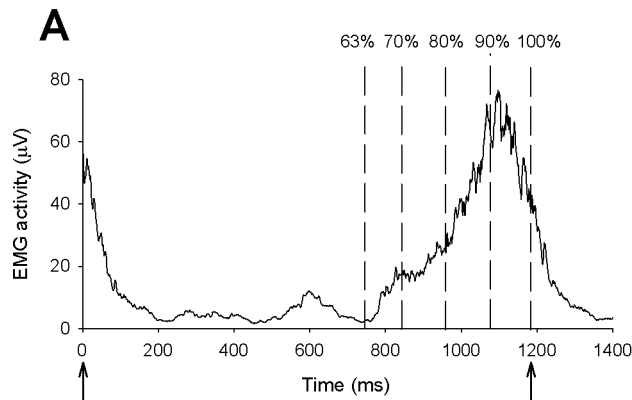


Figure 3-8

Raw EMG traces of the cSOL following stimulation of the iTN at 85% M-max at different phases of the gait cycle ($n = 1$). The black line represents the control step and the grey line represents the stimulated step. *A*: a normal EMG trace over the walking cycle with the location of the stimulus of the ipsilateral leg indicated. The arrows below the graph indicate heel strike. *B-E*: (with truncated axis) demonstrates stimuli delivered at 63 (*A*), 70 (*B*), 80 (*C*), 90 (*D*) and 100% (*E*) of the gait cycle. The vertical long dashed line indicates iTN stimulation, the vertical dotted lines represent the analysis windows. Each trace is the mean of 30 trials.

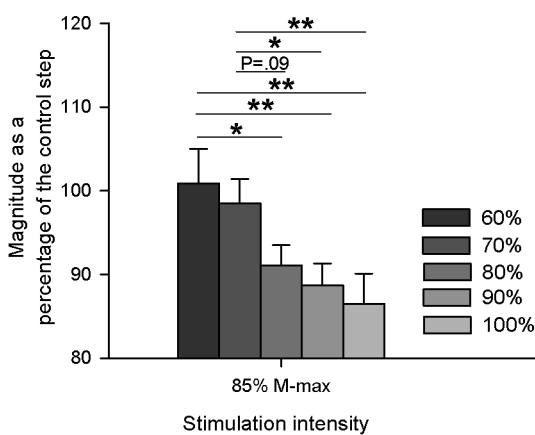
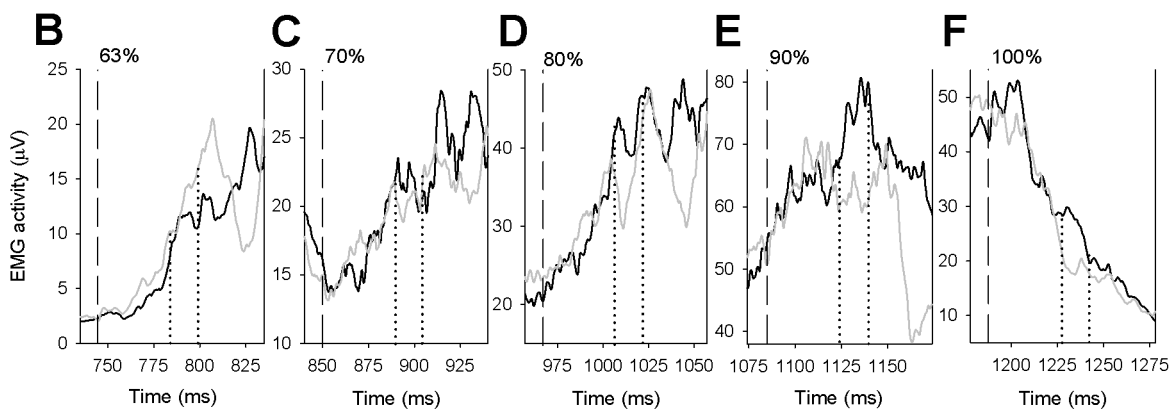


Figure 3-9

Mean \pm SEM for the magnitude of the response as a percentage of the control step over the age appropriate time windows for 85% M-max stimulated at 60, 70, 80, 90 and 100% of the gait cycle. *, ** and *** represent significant differences to $P < .05$, $P < .01$ and $P < .001$, respectively. Near significant results are reported with the level of significance.

3.5.2. Following stroke: During sitting

The Welch ANOVA revealed significant differences comparing CHR (P–NP and NP–P), SA (P–NP and NP–P) and HCs ($P < .05$). Post-hoc tests revealed significant differences between CHR (P–NP) and CHR (NP–P) vs. HCs ($P < .01$ and $P < .05$, respectively) and no significant differences between SA (P–NP) and SA (NP–P) vs. HCs ($P > .05$). The one-way ANOVA between patient groups and HCs for MT and 35% M-max revealed no significant differences ($P > .05$). Figure 3-10(A–D) displays the raw cSOL traces following stimulation of the iTN at 85% M-max for two HCs (Fig. 3-10A), one CHR patient (Fig. 3-10B) and two SA patients (Fig 3-10C and D). Two SA patients have been included due to the variability of responses in SA patients. Fig. 3-10(A–D) shows that the inhibitory response is more prominent in the HCs (Fig. 3-10A) than the CHR patient (Fig. 3-10B) and that the responses in SA patients are variable [Fig. 3-10(C and D)]. The SA patients shown represent the variability of the responses in SA patients [SA patient 1 (P–NP) shows a prominent inhibition; SA patient 2 (NP–P) shows a prominent facilitation].

The Levene test (comparing all groups) revealed significantly larger variability in the magnitude of the responses (with the iTN stimulated at 85% M-max) in the SA patients (both P–NP and NP–P) compared to CHR patients (both P–NP and NP–P) and HCs ($P < .001$). Figure 3-11(A–D) demonstrates the magnitude of the response in SA patients (Fig. 3-11A; P–NP and NP–P), CHR patients (Fig 3-11B; P–NP and NP–P) and HCs (Fig. 3-11D) stimulated at 85% M-max. In addition, the magnitude of the response as a function of the time since the lesion onset (Fig 3-11C) is shown for the P–NP and NP–P. Table 3-2 displays the results of the Levene tests performed post-hoc (on individual groups). Figure 3-11(A–D) suggests and table 3-2 shows significantly more heterogeneous responses between SA patients (both P–NP and NP–P) vs. CHR patients (both P–NP and NP–P) and HCs (post-hoc tests; $P \leq .05$).

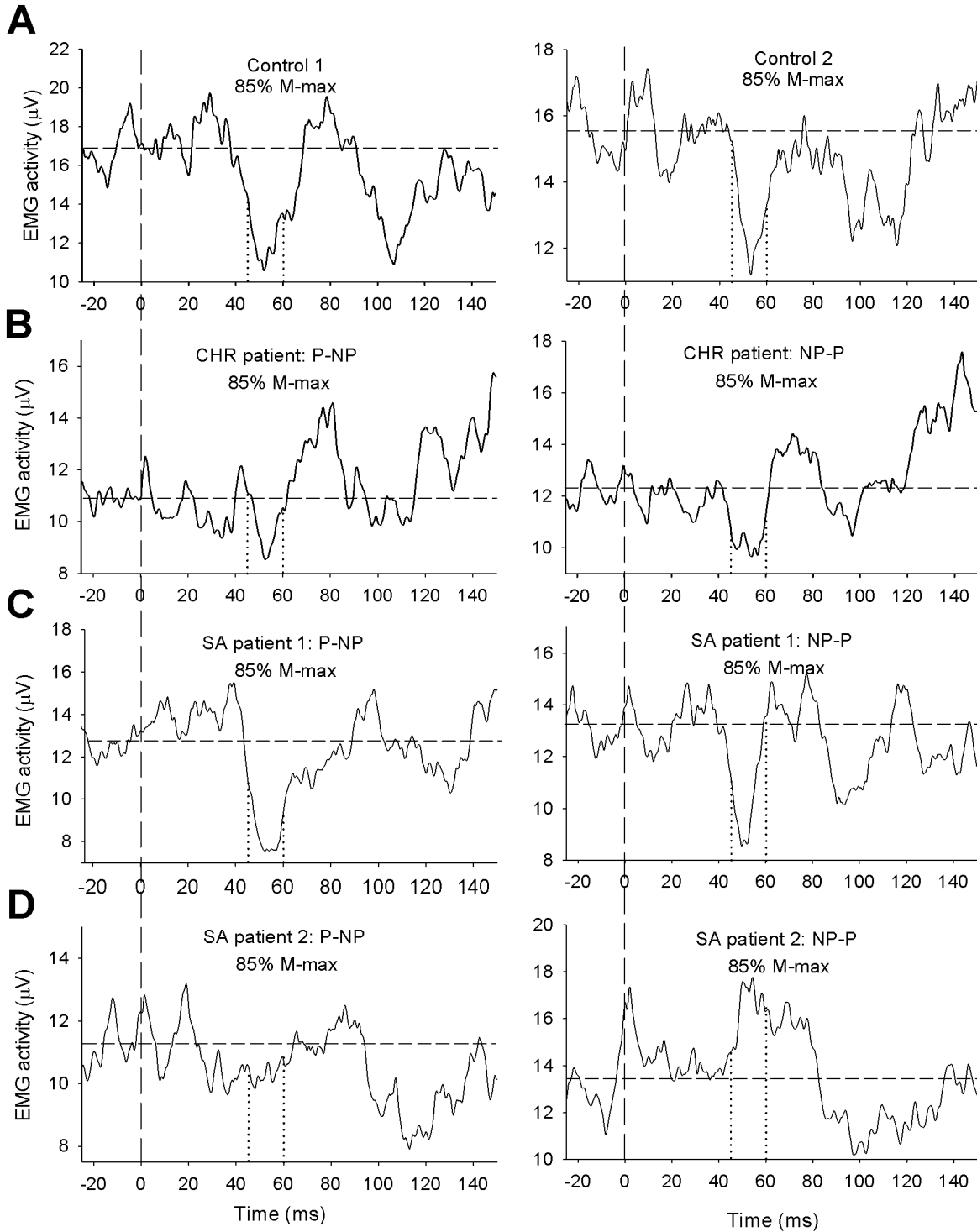


Figure 3-10

A–D: Raw cSOL EMG traces following stimulation of the iTN at 85% M-max of the iSOL for two control subjects (A), one CHR patient (P–NP and NP–P; B) and two SA patients (P–NP and NP–P; C and D). The vertical dashed lines indicate stimulation of the iTN and the vertical dotted lines represent the analysis window. The horizontal dashed line represents the approximate baseline. Each trace is the average of 30–45 recordings.

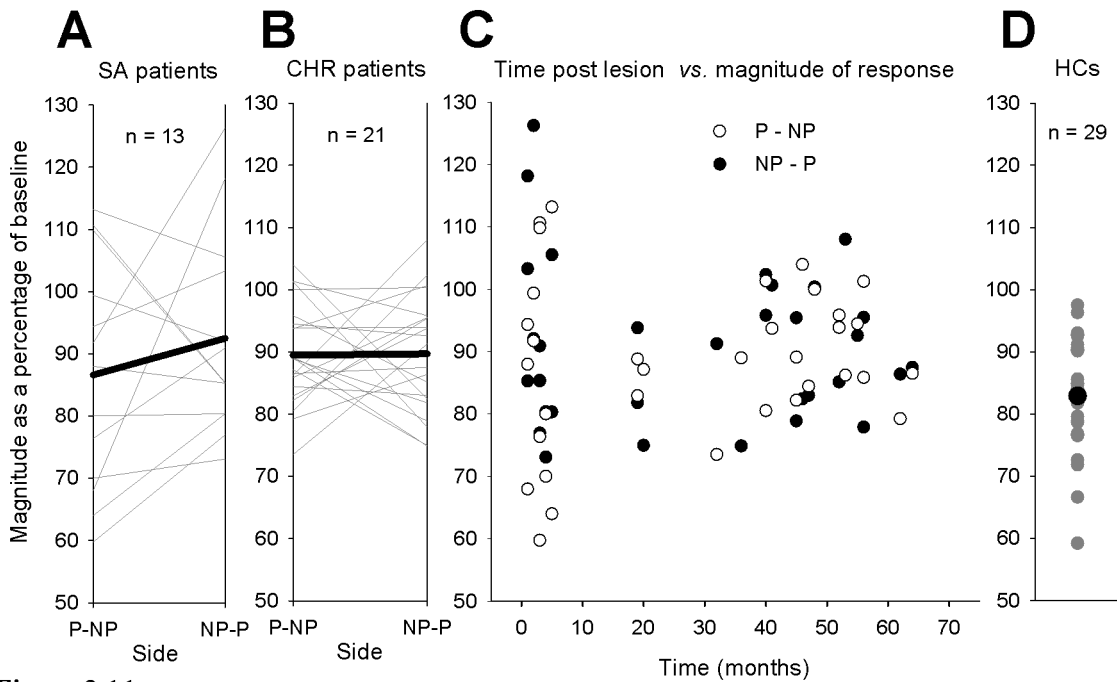


Figure 3-11

A–D: Magnitude of the response as a percentage of the baseline EMG activity for iTN stimulation at 85% M-max for SA patients (P–NP and NP–P; A and C), CHR patients (P–NP and NP–P; B and C) and HCs (D). A and B: The thin grey lines represent individual patients and the thick black line represents the mean. C: Magnitude of the response as a function of time since the lesion onset for the P–NP (white filled circles) and NP–P (dark filled circles). D: The grey dots represent individual subjects and the black dot represents the mean.

Table 3-2: Table showing the differences between groups following Levene tests conducted post-hoc

Control				
SA P-NP	P<.001			
SA NP-P	P<.05	n.s		
CHR P-NP	n.s	P<.001	P<.05	
CHR NP-P	n.s	P<.01	P=.05	n.s
Control	SA P-NP	SA NP-P	CHR P-NP	CHR NP-P

The repeated measures ANOVA comparing MT, 35% M-max and 85% M-max revealed significant differences for the HCs ($P < .001$), CHR (P–NP; $P < .05$), near significant differences for CHR (NP–P; $P = .058$) and no significant differences between stimulation intensity for SA (P–NP) or SA (NP–P; $P > .05$). The results of post-hoc tests are indicated in figure 3-12(A–E).

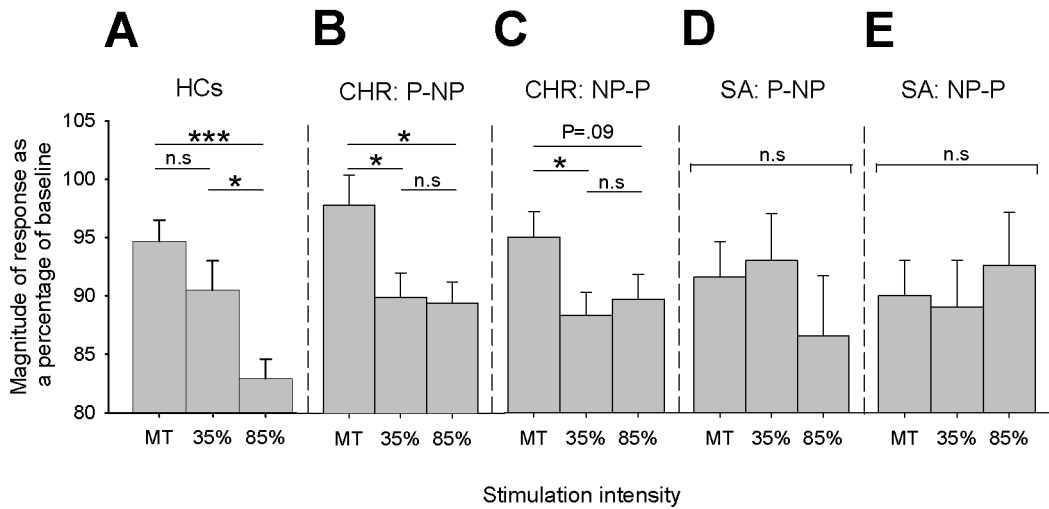


Figure 3-12

A–E: Mean \pm SEM of the magnitude of the response as a percentage of baseline comparing stimulus intensities (MT, 35% M-max and 85% M-max) for HCs (A), SA (P–NP; B), SA (NP–P; C), CHR (P–NP; D) and CHR (NP–P; E). *** and * represent significant differences to $P < .001$ and $P < .05$, respectively. Near significant differences are indicated. ‘n.s.’ represents non-significant results.

3.5.3. Following stroke: During walking

Figure 3-13(A–C) shows raw cSOL EMG traces following stimulation of the iTN at 85% M-max at approximately 90% of the gait cycle for one HC (Fig. 3-13A) and one patient [P–NP (Fig. 3-13B) and NP–P (Fig. 3-13C)]. The HC (Fig. 3-13A) and the patient (NP–P; Fig. 3-13C) display inhibitory responses however the patient (P–NP; Fig 3-13B) displays no inhibitory response (in the defined time windows).

One-way ANOVA and Levene tests revealed no significant differences in the magnitude or variability of the response between SA (P–NP), SA (NP–P), CHR (P–NP), CHR (NP–P) and HCs. As there were no differences in the variability or magnitude of the responses between SA patients, CHR patients and HCs, the patients were pooled (into P–NP and NP–P extremity). Following this, t-tests revealed significant differences in the magnitude of the response between P–NP vs. HCs (for 85% M-max; $P < .05$) and no significant differences between NP–P vs. HCs or P–NP vs. NP–P (additionally there were no significant differences in the variability). Figure 3-14 represents the mean \pm SEM of the responses for the HCs and patients (P–NP and NP–P) and indicates the location of the significant differences.

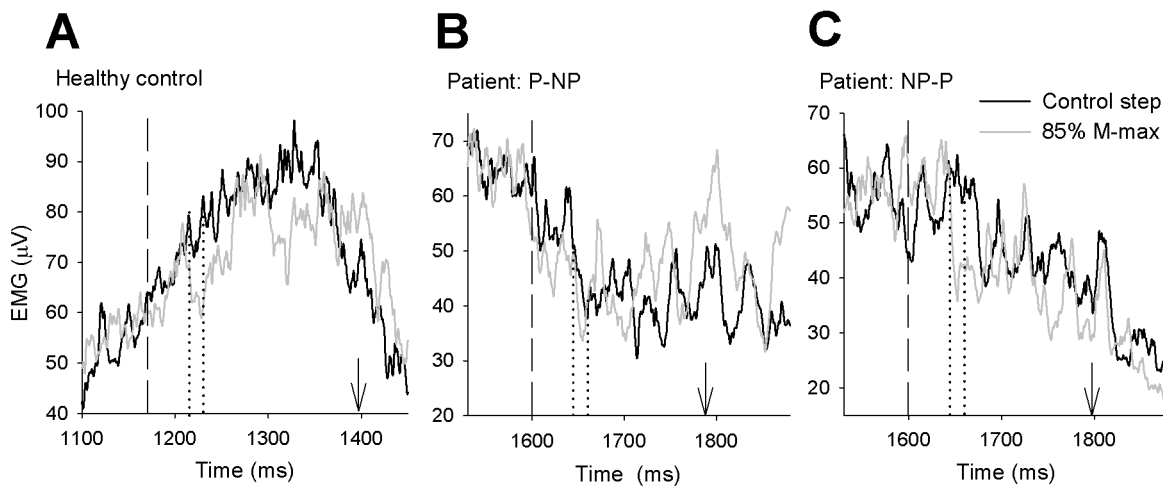


Figure 3-13

A–C: Raw (truncated) EMG traces for the cSOL with an average of 30–45 trials for one HC (A) and one patient P–NP (B) and NP–P (C). The black line represents the cSOL EMG with no stimulus and the grey line represents the cSOL following stimulation of the iTN at 85% M-max. The vertical long dashed lines represent stimulation of the iTN, the vertical dotted lines represent the analysis windows and the arrows indicate heel contact.

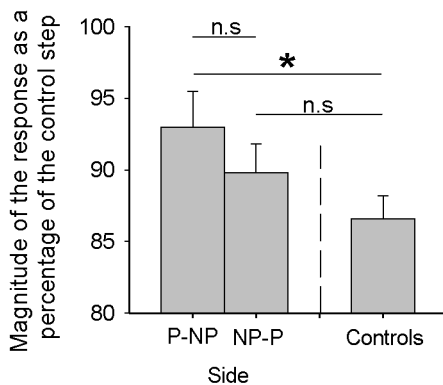


Figure 3-14

Mean \pm SEM of the magnitude of the response (in the defined time window) as a percentage of the control step for HCs ($n = 21$) and patients ($n = 21$; P–NP and NP–P). *, represents significant differences to $P < .05$. 'n.s' represents non-significant results.

3.6. Clinical measurements, medication status and lesion site

There was no significant relationship between the age of the subject, Fugl-Meyer lower limb score, 10 meter walk time or Ashworth scale vs. the magnitude of the response with the iTN stimulated at 85% M-max for P–NP and NP–P during sitting and walking ($P > .05$).

Only posterior circulation infarct (POCI) and partial anterior circulation infarct (PACI) lesion territories were compared due to the low number of patients with lacunar infarct (LACI) and total anterior circulation infarct (TACI) lesion territories. There were no significant differences in the magnitude and variability of the response and the lesion territory [POCI (P–NP and NP–P) and PACI (P–NP and NP–P)] for 85% M-max during sitting and walking ($P > .05$).

During sitting for the SA (P–NP), unpaired t-tests revealed that patients taking selective serotonin reuptake inhibitors (SSRIs) had significantly less prominent inhibitory responses than patients taking no medication ($P < .05$). There were no significant differences between patients taking SSRIs vs. patients taking no medication for sitting [SA (NP–P), CHR (P–NP), CHR NP–P], or walking (all groups; $P > .05$).

4. DISCUSSION

The main objective of this PhD was to investigate if there were short-latency crossed spinal pathways between ankle extensors in the human lower limb. A short-latency crossed spinal inhibition from the iTN (stimulated at the popliteal fossa) to the cSOL was observed. The inhibition became more prominent as the stimulation intensity increased, was not caused by ipsilateral cutaneous or CPN afferents, and was partly mediated by large diameter ipsilateral muscle/tendon afferents. It is likely that supraspinal projections modify/modulate the response as there was a phase dependence of the response and an impairment of the response following supraspinal lesions. From the results of this thesis it seems likely that comINs with group I or II (muscle/tendon) afferent input exist in the human

4.1. Possible nerve fibres involved

The short-latency crossed spinal inhibition became more prominent as the stimulation intensity increased. The response became evident at approximately $1.2 \times \text{MT}$, ischemia delayed the time of the minimum value and stimulation of the MpN and SuN were not a source of the cSOL inhibition. Also, as the H-reflex was significantly depressed at an ISI of 3 ms (although it should be noted that using the H-reflex underestimates the latency of the central delay; Pierrot-Deseilligny and Burke 2005: p. 9) it is likely that large diameter afferents with (possibly) 2–4 synapses mediate the initial inhibition which may then be augmented by slower afferents or more polysynaptic spinal pathways.

Unmyelinated/smaller diameter afferents: It is unlikely that unmyelinated or smaller myelinated afferents are a source of the response. This is due to the short-latency of the inhibition and the onset of the inhibition at stimulus intensities of approximately $1.2 \times \text{MT}$ (which is below the threshold of activation for these afferents).

Afferents of the iCPN: Due to the high stimulation intensities used to elicit a response in the cSOL it is possible that afferents from the iCPN mediate the response. From the results this is unlikely as stimulation of

the iCPN showed no cSOL responses at any stimulus intensity [when analyzed over the same time window as the iTN to cSOL (using the RMS window)].

Cutaneous afferents: As stimulation of the SuN and MpN at any stimulation intensity at the ankle demonstrated no short-latency inhibition (even accounting for the distal stimulation site), it is unlikely that these mainly cutaneous afferents are a source of the response. Neither Duysens et al. (1991), Delwaide et al. (1981) nor Burke et al. (1991) who stimulated the SuN and/or the inferior tibial nerve reported contralateral inhibitory responses at the latencies observed in the current thesis. Despite this, as the iTN was stimulated at the popliteal fossa and the cutaneous afferents were stimulated at the ankle, it is possible that cutaneous afferents between the ankle and the popliteal fossa, that would not have been stimulated with cutaneous nerve stimulation at the ankle, are a source of the response. Also, the cutaneous afferents directly under the stimulation site in the popliteal fossa (due to the high stimulation intensities required to elicit a prominent inhibition) could be a source of the response. This possibility could be eliminated as high stimulation intensities of the iCPN did not show a response in the cSOL but as there can be a site dependence of ipsilateral and contralateral responses (for nociceptive and cutaneous afferents stimulated at the sole of the foot; Spaich et al. 2004, 2006, 2009) it is still plausible that cutaneous afferents around the knee are a source of the response. Although the electrodes could be shifted to stimulate the cutaneous afferents under the stimulation electrodes, due to the high stimulation intensities used and the close proximity of the nerves in the popliteal fossa it is unlikely that only the cutaneous afferents under the stimulation electrodes would be stimulated.

Group I and group II (muscle) afferents: The results of the current thesis suggest that either group I or group II afferents could be a source of the inhibitory response. The ischemic block significantly delayed the time of the minimum of the inhibition however no changes were observed in the magnitude, onset or duration of the inhibition. As the application of ischemia would eliminate (large diameter) group Ia and Ib afferents, it shows that these afferents may partly be a source of the response. One subject showed an elimination of the

response during ischemia and therefore, for only this subject, group I afferents could be inferred. As there is some overlap of the diameter of group I and II afferents it is possible that ischemia also blocked some group II afferents. Therefore, as ischemia only partially altered the inhibition it is possible that group I and/or group II afferents could be a source of the response.

In **Study I**, the average stimulation intensity for the onset of the inhibition was $1.2 \times \text{MT}$. Three subjects showed contralateral responses at stimulation intensities below $1 \times \text{MT}$ and therefore it is possible that for these subjects group I afferents are a source of the response. However, group I afferents have been recruited at stimulation intensities up to $4\text{--}5 \times \text{MT}$ (in the TA; Gracies et al. 1994) and therefore higher threshold group I afferents could be a source of the response in other subjects. With the additional recruitment of group I afferents at high intensities there is also the additional recruitment of group II afferents.

In the cat crossed responses via comINs have been observed from group I and II afferents (see *sections 1.1.1 and 1.1.2*). Contrary to the findings in the cat, that displayed minimal inhibitory responses from the SOL/GAS nerve (Arya et al. 1991; Holmqvist 1961; Perl 1958), in the current thesis an inhibitory response was observed in the cSOL following stimulation of the iTN in most healthy subjects [this could be due to the differences between the human and the cat (see *section 1.5*)]. Arya et al. (1991) reported primarily inhibitory responses to the cMN following stimulation of group II afferents regardless of the ipsilateral nerve stimulated (flexor or extensor) or the cMN assessed however, other studies have reported inhibitory and/or excitatory responses in the cMN following stimulation of group I and/or II afferents (For example, Harrison et al. 1986; Jankowska and Noga 1990; Jankowska et al. 2005a, 2006, 2008, 2009). Although Arya et al. (1991) provides evidence for group II afferents as a source of the response, the other studies provide evidence for group I, group Ib and/or group II afferents. As some studies in the cat have reported that group Ia afferents provide the initial contralateral response, followed by group Ib afferents (probably followed by group II afferents; at higher stimulation intensities), it is possible that in the human, group I afferents mediate the initial part of the inhibition while group II afferents (or polysynaptic pathways) mediate the later parts. Another possible

explanation [(proposed by Edgley et al. (2004)], is that interactions between group I and group II afferents and pre-synaptic modulation may ‘hide’ IPSPs and EPSPs. When higher electrical stimulation intensities are administered, these ‘hidden’ connections maybe exposed resulting in an inhibition of the cSOL EMG.

Contralateral motor efferents: Some studies in the human have reported differences in the sign of responses of small and large diameter motor efferents (see Nielsen and Kagamihara 1993; Pierrot-Deseilligny and Burke 2005: p. 425). As greater pre-contraction levels of the cSOL showed no difference in the magnitude of the response (and the cSOL H-reflex showed a comparable inhibition to the pre-contracted cSOL) it is likely that large and small motor efferents can be inhibited. Additionally, the results suggest that synchronously evoked (non-voluntary) motor volleys can be inhibited in the same manner as supraspinally induced asynchronous (voluntary) motor volleys.

4.2. Differences between iTN to cSOL and the other investigated nerves/muscles.

In the human there is not a non-selective short-latency inhibition (which might be expected from research in the cat: see Arya et al. 1991), as stimulation of other nerves–muscles [in sitting; iTN–cTA, iCPN–cTA or iCPN–cSOL (**AdExp**)] displayed no inhibitory responses at the latencies observed from the iTN–cSOL (although longer latency responses at possible spinal onset latencies were observed). It is possible that this is due to the differential control of lower limb flexors and extensors in the human (Brouwer and Ashby 1992). Brouwer and Ashby (1992), using transcranial magnetic stimulation (TMS), noted that in the pre-contracted muscle, 100% of subjects had responses in the TA whereas < 30% had responses in the SOL (of which most were weak). The stronger responses in the ankle flexors compared to extensors (for the GAS) was also apparent during walking (Schubert et al. 1997). Dietz (2003) stated that direct projections from the corticospinal tract have a large role in the control of flexors however, proprioceptive feedback is more important in the control of extensors. The current thesis suggests that short latency spinally mediated

interlimb proprioceptive feedback is strongly coupled between extensors and weakly coupled to or from flexors. This might indicate/confirm that the proprioceptive feedback is more important in the control of extensors than flexors.

4.3. Supraspinal modulation of the response

The short-latency crossed inhibitory response was modulated by the phase of the gait cycle and became more prominent towards the swing to stance transition. It was also significantly less prominent in CHR patients compared to HCs, significantly more variable in SA patients compared to CHR patients and HCs and less prominent from the P–NP extremity in patients during walking compared to HCs. These results suggest that the short-latency crossed spinal inhibitory response is modulated by supraspinal areas.

The modulation of the response likely occurs at the pre-motor neuronal level as there was a phase dependence of the response, and even when there were matched levels of EMG (100% of the gait cycle compared to 70% of the gait cycle; see figure 3-8), an inhibition was observed. This suggests that the response is modulated by supraspinal input and not only modulated by the increased excitability of the α -MN pool. Also, as increasing the cSOL contraction level from 5–15% to 15–30% of the MVC (during sitting) showed no change in the response magnitude as a percentage of baseline, it is likely that the differences between 60 and 70% vs. 80, 90 and 100% of the gait cycle are due to pre-motor neuronal factors. Further support for pre-motor neuronal and supraspinal modulation of the response was the abnormal responses in the patients (most of whom had limited spasticity). It is proposed that reflex pathways are altered due to spasticity; such as reduced reciprocal inhibition (Crone et al. 1994, 2003, 2004) and post activation depression (Grey et al. 2008; Lamy et al. 2009). Due to the lack of relationship between response magnitude and spasticity in the current thesis it is possible that the alterations are due to descending projections from supraspinal areas and not spasticity.

The responses in SA and CHR patients were affected bilaterally. This dysfunction in crossed spinal reflex pathways shows the possibility that the responses are modulated by bilateral descending projections to comINs, INs and/or MNs. Extremities ipsilateral and contralateral to the lesion in stroke patients can be affected (Khaja and Grotta 2007) which is likely due to ipsilaterally and contralaterally projecting descending pathways (Jankowska and Edgley 2006). In the cat, ipsilateral and/or contralateral supraspinal areas project to comINs, including monosynaptic connections from the vestibulospinal tract, mono/di/trisynaptic connections from the reticulospinal and rubrospinal tracts and di/trisynaptic (and probably more polysynaptic connections) from the corticospinal tract, cuneiform nucleus, and MLR (see *section 1.1.4*). With these factors considered, it seems likely that bilateral descending projections could affect the response (however the exact connections/descending pathways are unknown).

Determining the supraspinal areas involved in the modulation the response from the current thesis is difficult. Although the best indication would be to observe the responses in relation to the lesion site, no patterns were established. This is probably due to the classification of the lesion site. The Bamford classification (Bamford et al. 1991) determines the arterial territory based on the clinical presentation of the patient immediately following the lesion. Therefore, only territories (TACI, PACI, POCI or LACI) are determined and the exact lesion site (and extent of the lesion) is unknown. The four groupings of the Bamford classification are probably too general to observe an effect, as the control of comINs are probably modulated by (or via) lower brainstem structures with numerous inputs from all regions of the brain. Although, computer tomography scans were performed within 24 hours of the lesion onset for some subjects, it was impossible to determine the lesion location or the extent of the lesion from these. These are performed routinely following admission to the hospital [to exclude (for example) haemorrhage or tumours] and were not performed to assist in the identification of the lesion site for the current thesis.

Although SA and CHR patients showed impaired modulation by supraspinal areas, there were differences in impairments between the patient groups (SA patients had an increased variability and CHR patients had a

reduction in the prominence of the response). Immediately following stroke, there are large changes to supraspinal centres (Nudo 1999, 2006). This includes ipsilaterally and contralaterally '*GABA_A receptor down regulation, NMDA receptor enhancement, neuronal hyperexcitability, synaptogenesis (only after day 18, contralaterally)*', ipsilaterally only '*decrease and/or increased dendritic branching, decreased spine density, neural sprouting and alterations to motor maps*' and contralaterally only '*increased cortical thickness, dendritic growth (up to day 18 post lesion), dendritic elimination (after day 18 post lesion) and increased spine density (after day 18 post lesion)*' (Nudo 1999). As these changes subside (after three to six months), the system becomes more stable which results in more stable responses (stated by Brown 2006; Skilbeck et al. 1983; Swayne et al. 2008). Swayne et al. (2008) reported large intra/inter subject variability in responses within/from cortical areas in acute and SA patients following stroke. It was proposed that this variability was due to the reorganisation of cortical networks. Therefore, the increased variability observed in SA patients in the current thesis (but not the CHR patients) is possibly due to the instability in brain areas.

Interestingly, during walking there were no differences in the variability of responses between SA and CHR patients. There are a number of possible reasons for this. The SA patients in sitting had a reduced time between the lesion onset and testing. During sitting, 85% of SA patients had lesions ≤ 4 months old whereas during walking only 29% had lesions ≤ 4 months old. As cortical responses become more stable over time (Swayne et al. 2008) it is possible that some of the SA patients (in walking) had responses that had stabilised. Due to the limited number of SA patients in the walking study it is possibly [but unlikely (as no SA patients displayed abnormal variability)] due to type II statistical error. Also, 16 patients participated in both the sitting and walking protocols. These were performed on separate days (ranging from two days to seven months between experiments). The sitting experiments were always performed on the first day. Due to this, the patients may have become more comfortable with the testing procedures during the walking experiments. As there seems to be supraspinal modulation of the response, this could have changed descending projections, focus (Malone and Bastian 2010) and comfort during testing, which might have made the results

more stable. However, as t-tests and Levene tests comparing the patients that had previously performed sitting experiments vs. patients that had not previously performed experiments revealed no significant differences ($P > .05$), it is unlikely that this is the cause of the difference. Another possible explanation is that there is an alteration to the descending projections from supraspinal areas when walking compared to sitting. Walking is a functional task and sitting (pre-contracting the muscle) is an unnatural task. The automation of the task may have been more natural for the patient and therefore the responses could be less variable. There are indications that the response might be affected by 5-HT (however we are uncertain about the weight of this finding). In sitting, from the P–NP extremity, SA patients taking SSRIs had significantly less prominent responses than patients taking no medication. From the NP–P extremity, patients taking SSRIs had, on average, less prominent responses than patients taking no medication (however, this was non-significant). There were no differences due to medication status in the prominence of the response in CHR patients during sitting or CHR and SA patients during walking. Although, this may indicate that 5-HT affects comIN responses in the human spinal cord, this conclusion should be treated with caution. It seems unusual that SSRI administration was only significantly altered from the P–NP in SA patients during sitting. It would be expected if 5-HT had a role in the modulation of the response, that a significant reduction in the prominence of the response would be evident in SA patients from the NP–P extremity, and CHR patients during sitting and CHR and SA patients during walking. It is possible that it is due to type I statistical error and is a ‘false positive’ result. However, if it is not a ‘false-positive’ result it might indicate that 5-HT has an effect on the response in the human. In the cat, tonic descending drive from the dorsolateral funiculus mediated by 5-HT receptors had an effect on crossed spinal responses (Aggelopoulos et al. 2006). With supraspinal connections intact, or unilateral transection of the either the ipsilateral or contralateral dorsolateral funiculus, the responses in the cMN following ipsilateral group II afferent stimulation were inhibitory. Following bilateral transection of the dorsolateral funiculi the responses in the cMN were excitatory. These became inhibitory following the administration of a 5-HT agonist and remained excitatory

following administration of a 5-HT antagonist. Additionally, ipsilateral lamina VIII comINs with monosynaptically excited by the reticulospinal tract had facilitated cMN responses following the administration of 5-HT agonists (Hammar et al. 2004). The cats that were used in these investigations were acutely spinalised and CHR effects were not investigated. Therefore, SA patients may have more characteristics in common with the acutely affected cat than the CHR patients. There might be other explanations for the result such as changes to cortical interactions, spinal interneuronal excitability and/or descending drive from supraspinal regions, however these reasons are speculative and cannot be confirmed.

4.4. Functional significance and considerations

It is difficult to ascertain the functional significance of the response. The responses were evoked by electrical stimulation and not a physiological stretch of the muscle. By applying electrical stimulation there is an artificially produced, temporally summated, synchronous afferent volley from the TN. This differs from the asynchronous volleys elicited by a physiological stretch of the muscle. An asynchronous volley may not have the impetus to depolarise the INs involved in the crossed spinal response. Additionally, Morita et al. (1998) demonstrated that ipsilateral spinal reflexes elicited by electrical stimulation are processed differently than a tendon tap. Therefore, although functional implications are discussed below the method of stimulating the iTN afferents should be considered.

Due to the phase modulation of the response and the changes in the response in patient populations during sitting and walking it is possible that the response has some functional significance. The short-latency crossed inhibitory response became more prominent towards the swing to stance transition of the ipsilateral leg for 85% M-max and 35% M-max (while the contralateral leg was in push off). A sudden plantarflexion of the swinging foot while the foot is supposed to be dorsiflexing indicates a threat to the stability of the body. The threat to balance is greatest if this occurs at the gait transition phase. It is hypothesized that the large synchronous afferent volley to the spinal cord signals a mechanical disturbance to the iSOL. Therefore, an

inhibitory response in the cSOL EMG activity could be a method to halt the forward progression of the contralateral leg (in push off) toward the source of the disturbance. Studies on ipsilateral reflexes, have noted the importance of gait transition phases from stance to swing [for example, Andersen and Sinkjær 1999 (for H-reflex but not stretch reflex modulation); Capaday and Stein 1987] or swing to stance phase (for example, Baken et al. 2005; Dietz et al. 1986; Duysens et al. 1991; Faist et al. 1999) showing more prominent responses at these times of the gait cycle. The current thesis indicates that the phase transition of the ipsilateral leg could be important for the expression of the inhibition. However, as the phase transition of the ipsilateral leg from stance to swing was not investigated (as there was no cSOL EMG activity to show an inhibition) it is difficult to ascertain if the transition phase from stance to swing is also important for the response modulation. Previous studies have demonstrated an increase in the magnitude of responses (through H-reflex modulation) through stance peaking at the end of the stance phase (Andersen and Sinkjær 1999; Capaday and Stein 1987) therefore it is possible that the increased inhibition of the cSOL responses in late stance was due to pathways projecting to the contralateral leg. As the responses were significantly affected bilaterally (in patients during sitting) and unilaterally (from the P–NP extremity, during walking) it cannot be confirmed that the response is due to only descending projections to the ipsilateral leg.

There have been a number of other proposals to the functional purpose of short-latency crossed spinal responses. The inhibition may be a method to reduce the EMG activity of the contralateral leg until supraspinal pathways have time to act to voluntarily and appropriately modify the EMG activity (suggested by Frigon and Rossignol 2008). In the cat, studies have suggested that these short-latency inhibitory pathways may provide interlimb communication when contralateral limb extension would be inappropriate and that the response may be a spinally mediated way of synchronizing the EMG of the two legs (Arya et al. 1991; Frigon and Rossignol 2008). With these explanations in mind, the inappropriate responses in the cSOL of patients could indicate an inability to appropriately coordinate the legs following a mechanical disturbance to the ipsilateral limb. This may have implications to the regaining of balance or the inability to quickly avoid

obstacles. This may in turn be a contributor to the increased incidence of falls in patient populations (Jorgensen et al. 2002; Mackintosh et al. 2005).

It should be noted that not all healthy subjects displayed inhibitory responses and some subjects displayed very small inhibitory responses at 80, 90 and 100% of the gait cycle (although the overall effect was a significant inhibition). In addition, the percentage of the gait cycle in which the inhibition commenced varied between subjects. This could be explained by subjects employing different walking strategies causing an alteration to the reliance on feedback from the ankle extensors. This could direct descending projections from supraspinal centers to pre-synaptically inhibit or excite comINs or INs mediating this response. This may result in a reduced (or increased) expression of the crossed response at different phases of the gait cycle. The magnitude of the reflex could be altered based on the requirement of the subject for the reflex. An increased or reduced requirement for the short-latency crossed response may cause an up or down regulation of the response in some subjects.

The response assessed in the current thesis is only one response/pathway mediating interlimb coordination. The inhibitory response was often followed by longer latency inhibitory or facilitatory responses from the iTN–cSOL. Also, although inhibitory responses were not observed from the iTN–cTA, iCPN–cSOL and iCPN–cTA, some subjects displayed longer latency responses from these muscles that had response onset latencies that might be spinally mediated (**AdExp**). As the SOL is only one muscle involved in gait, larger muscle groups over the knee and hip may have a greater role in the control of interlimb coordination.

Therefore, although the response (over the defined time windows) was significantly inhibitory (during sitting and walking) it may not have been large enough to affect function (due to the other muscles in the lower limb; and that statistical significance does not always mean functional significance).

The lack of relationship to functional measures in the patients may indicate the response is not functional. However, 1) For the Ashworth scale, the limited number of patients with severe spasticity reduced the power of the statistical tests so that there may not have been an effect 2) The clinical tests may not have been

sensitive enough to observe differences in the response 3) The tests may have had a ceiling effect (as with the Fugl-Meyer assessment; Gladstone et al. 2002) 4) the tests may not have been the correct tests to observe an effect. The Berg Balance Scale or sway index (during walking) may have been more appropriate tests.

4.5. Future directions

This thesis demonstrates that SA patients have more variable responses than CHR patients. For this reason it would be interesting to follow patients longitudinally from the acute to CHR phase, testing patients many times over these periods. It is possible that the variability in SA patients is due to day-to-day variability i.e. One day patients display large facilitatory responses and the next they show large inhibitory responses. As this has been reported in cortical responses (Swayne et al. 2008) it is possible that this will also occur in this spinal response.

Although an attempt was made in this thesis to demonstrate the role of supraspinal areas to the control of the response (through the use of stroke patients) and it seems likely that these have a role, it is difficult to ascertain the exact importance/role of these in the control of the response. To identify the general role of supraspinal areas, the response could be observed in patients with complete spinal cord or diffuse brain injury, as by completely removing descending projections, the role of supraspinal areas can be assessed.

Study II provides a method to investigate patients with no/minimal descending drive.

Identifying the exact supraspinal areas is difficult. Non-invasive measures such as transcranial electrical stimulation, TMS, repetitive TMS and transcranial direct current stimulation can be used to modulate the excitability of cortical areas and/or test the importance of corticospinal projections. However, as there seems to be limited direct cortical projections to ankle extensors in the human and reduced corticospinal projections to comINs in the cat it is possible that other methods will be required to assess the dominance of other brain areas. This is difficult to assess, as no methods have been developed to focally remove non-cortical brain areas. Imaging or hypnosis could be used to assess the degree that certain brain areas modulate the response.

However, this may be complex and the complexity itself may alter the projections to comINs. Although, direct removal/stimulation of lower brain areas such as the red nucleus, and reticulospinal tract are not possible in the human, investigation of these areas in the primate (and possible invasive studies on comINs in the spinal cord) could provide insight into the control of comINs in an animal more evolutionarily related to the human. This may increase the transference of results to the human.

There are a number of studies that can be performed to elucidate on the spinal pathways involved in the response. These include vibration of the ipsilateral leg, intake of certain neurotransmitter agonists/antagonists (such as 5-HT, NA, tizanidine, benzodiazapines, L-DOPA), convergence of reciprocal inhibition from the TA on to INs involved in the response, heteronymous and homonymous inhibitory/excitatory convergence, the role of pre-synaptic inhibition and the possible mediation of the response by Renshaw cells (which may be important; see Nishimaru et al. 2004).

4.6. Conclusions

From the current thesis it can be concluded that short latency interlimb reflexes are observed from the iTN to the cSOL and are (i) inhibitory and observed in sitting and a functional task as walking (ii) modulated by supraspinal areas due to the phase dependence and alterations in patients with stroke (iii) likely mediated by large diameter ipsilateral muscle/tendon afferents. The current thesis suggests that comINs are present in the human with input from ipsilateral muscle or tendon afferents of the triceps surae to motor efferents of the cSOL. Although it is difficult to propose the exact pathways, modulatory role of supraspinal areas and functional consequences of the response, this thesis provides the basis for future studies.

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6. APPENDIX

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